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Serum vitamin B_{12} and related 5-methyltetrahydrofolate-homocysteine methyltransferase reductase and cubilin genotypes predict neural outcomes across the Alzheimer's disease spectrum

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Abstract

Epidemiological studies show mixed findings for serum vitamin B_{12} (B_{12}) and both cognitive and regional volume outcomes. No studies to date have comprehensively examined, in non-supplemented individuals, serum B_{12} level associations with neurodegeneration, hypometabolism and cognition across the Alzheimer's disease (AD) spectrum. Serum B_{12} was assayed from the Alzheimer's Disease Neuroimaging Initiative (ADNI) and the Australian Imaging, Biomarker & Lifestyle Flagship Study of Ageing (AIBL). Voxel-wise analyses regressed B_{12} levels against regional grey matter (GM) volume and glucose metabolism ($P < 0{\cdot}05$, family-wise corrected). For ADNI GM, there were thirty-nine cognitively normal (CN), seventy-three mild cognitive impairment (MCI) and thirty-one AD participants. For AIBL GM, there were 311 CN, fifty-nine MCI and thirty-one AD participants. Covariates were age, sex, baseline diagnosis, *APOE4* status and BMI. In ADNI, higher B_{12} was negatively associated with GM in the right precuneus and bilateral frontal gyri. When diagnostic groups were examined separately, only participants with MCI, or above an established cut-off for cerebrospinal fluid (CSF) total tau showed such associations. In AIBL, higher B_{12} was associated with more GM in the right amygdala and right superior temporal pole, which largely seemed to be driven by CN participants that constituted most of the sample. Our results suggest that B_{12} may show different patterns of association based on clinical status and, for ADNI, AD CSF biomarkers. Accounting for these factors may clarify the relationship between B_{12} with neural outcomes in late-life.

Key words: **Nutrition: MRI: Biomarkers: Neuroimaging**

Deficient levels of vitamin B_{12} or folate lead to increased levels of homocysteine, which is a risk factor for thrombosis, microbleeds, strokes, cognitive impairment and neuronal atrophy[1,2]. Vitamin B_{12}, or cobalamin, normally contributes to the production of myelin in the central nervous system and fatty acid metabolism. Vitamin B_{12} is naturally occurring in meat, fish, milk and eggs[3]. When vitamin B_{12} is consumed, it first binds to haptocorrin (or transcobalamin I) to protect the vitamin B_{12} from stomach acid.

Abbreviations: AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; AIBL, Australian Imaging, Biomarker & Lifestyle Flagship Study of Ageing; Aβ, amyloid-β; CDR-sob, Clinical Dementia Rating sum of boxes; CN, cognitively normal; CSF, cerebrospinal fluid; CUBN, cubilin; FDG, fluorodeoxyglucose; FWE, family-wise error; GM, grey matter; MCI, mild cognitive impairment; MTHFR, methylenetetrahydrofolate reductase; MTRR, 5-methyltetrahydrofolate-homocysteine methyltransferase reductase; TCN2, transcobalamin II; WM, white matter.

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Intrinsic factor is produced in the stomach and binds to vitamin B_{12} in the intestines to allow absorption into the ileum enterocytes via the membrane protein cubilin (CUBN)[4,5]. Importantly, vitamin B_{12} is transported to the liver via transcobalamin II, where it takes place in folate/vitamin B_{12}-dependent remethylation to facilitate the conversion of homocysteine to methionine and the subsequent methylation of DNA, proteins and lipids[6].

The literature is currently mixed about the role of vitamin B_{12} in brain health and late-life adults with or without Alzheimer's disease (AD)-related cognitive impairment. On the one hand, vitamin B_{12} is significantly lower in both plasma[7] and cerebrospinal fluid (CSF)[8] of patients with AD *v.* normally ageing controls. Further, vitamin B_{12} deficiency in aged, cognitively normal (CN) adults with diabetes was associated with less grey matter (GM) volume in the left middle temporal pole and the left insula[9] suggesting that supplementation may be useful. Indeed, clinical trials have found that vitamin B_{12} supplementation may slow brain atrophy in mild cognitive impairment (MCI), which is often a precursor state to AD[10], when *n*-3 fatty acid levels are sufficiently high, perhaps by changing homocysteine levels[11,12]. On the other hand, aged adults with mildly elevated plasma homocysteine levels showed less total brain volume after 2 years of daily supplementation with 500 μg of vitamin B_{12} and 400 μg of folic acid *v.* placebo tablet[13]. This complication may in part be due to the *APOE4* carrier status, the strongest genetic risk factor for developing AD, which may modify associations between vitamin B_{12} and regional GM[14].

For cognitive function, the literature is also mixed regarding vitamin B_{12} supplementation efficacy or its use as a biomarker to track AD-related cognitive decline. Despite controversy[15] surrounding the meta-analysis by Clarke *et al.*[16] in selecting rigorous clinical trials and sensitive global cognitive measures in normal ageing, meta-analyses indicate that vitamin B_{12} supplementation may not influence cognitive decline among CN aged adults with type 2 diabetes[16], perhaps due to the mild nature of cognitive decline in normal ageing and difficulty in controlling for nutritional status[17]. Vitamin B_{12} combined with folate does appear to have modest clinical efficacy in CN or MCI participants, however[18]. Qin *et al.* found that individuals in the top quintile for vitamin B_{12} intake showed increased performance in working memory, but no differences in memory or executive function tests[19]. Thus, clinical status and perhaps underlying features of AD, such as amyloid-β (Aβ) and total tau, may modulate vitamin B_{12} supplementation. Although vitamin B_{12} in rodent models is protective against Aβ[20] and total tau fibrillar accumulation[21], it is not clear what vitamin B_{12} is tracking in terms of neural or cognitive outcomes when clinically significant levels of these AD hallmarks are already present in the brain.

Thus, wide variability in vitamin B_{12} associations with GM atrophy or fluorodeoxyglucose (FDG) metabolism may be due to vascular factors, clinical status, CSF levels of Aβ and total tau, and/or genetic methylation patterns specific to vitamin B_{12}[21–23]. Thus, beyond examining the main effects of vitamin B_{12} on neural outcomes of interest, we examined potential modulators such as: (1) the vascular risk marker homocysteine; (2) established cut-offs of Aβ and total tau accumulation relevant to AD[24]; (3) baseline clinical status and (4) SNP among four *a priori* selected genes involved in vitamin B_{12} transport, uptake, and metabolism, *CUBN*, methylenetetrahydrofolate reductase (*MTHFR*), 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (*MTRR*) and transcobalamin II (*TCN2*)[25]. To further elucidate conflicting findings, analyses were separately conducted in two large cohorts spanning North America and Australia that had similar MRI and cognitive data but different proportions of adults without impairment *v.* MCI or AD.

Materials and methods

Participants

Data from aged adults were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu) and Australian Imaging, Biomarker & Lifestyle Flagship Study of Ageing (AIBL) study group[26]. The ADNI was launched in 2003 as a public–private partnership, led by principal investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial MRI, positron emission tomography, other biological markers and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. For up-to-date information, see http://www.adni-info.org. Written informed consent was obtained from all ADNI participants at their respective ADNI sites. The ADNI protocol was approved by site-specific institutional review boards. Data were collected in accord with the Helsinki Declaration of 1975. To eliminate the influence of vitamin B_{12} supplementation, especially in participants who were receiving increased care from physicians, analyses only included participants who did not report taking vitamin B_{12} supplements or multivitamins, excluding 299 ADNI participants and seventy-one AIBL participants.

Serum biomarkers

Vitamin B_{12} data were downloaded at baseline for ADNI participants and obtained through a data request from AIBL. Vitamin B_{12} was assayed via a Siemens ADVIA Centaur XP autoanalyzer immunoassay by Quest Diagnostics as of 16 April 2008 for ADNI participants. For the AIBL participants, vitamin B_{12} was assayed by the Royal Melbourne Pathology in Melbourne and PathWest Laboratory Medicine WA in Perth via ADVIA Centaur Assay – competitive immunoassay. Blood processing took place between 2007 and 2014. Homocysteine in ADNI was obtained through the ADNI Biomarker Core, using a validated enzyme immunoassay methodology[27]. Homocysteine in AIBL was obtained through a data request, where all assays were conducted in two laboratories via MMULITE 2000 – competitive immunoassay. ADNI vitamin B_{12} levels are reported in pg/ml, and AIBL levels were reported from our data request in pmol/l. All AIBL vitamin B_{12} values were converted to pg/ml for consistency.

APOE *genotype*

The ADNI Biomarker Core at the University of Pennsylvania conducted *APOE* ε4 genotyping. *APOE* genotypic data were downloaded for AIBL participants. We characterised participants as

having zero *APOE4* alleles, one *APOE4* allele or two *APOE* ε4 alleles.

Amyloid and tau cerebrospinal fluid biomarkers

CSF sample collection, processing and quality control of p-tau, total tau and A$\beta_{1\text{-}42}$ are described in the ADNI1 protocol manual (http://adni.loni.usc.edu/) and Shaw *et al.*[24], where CSF A$\beta_{1\text{-}42}$ and total tau cut-offs were <192 and >93 pg/ml, respectively. Amyloid and tau markers were only available in a very small subset of AIBL participants, and so these were not assessed.

Neuropsychological assessment

Cognitive testing was available for both ADNI and AIBL. ADNI utilises an extensive battery of assessments to examine cognitive functioning with particular emphasis on domains relevant to AD. A full description is available at http://www.adni-info.org/Scientists/CognitiveTesting.aspx. All subjects underwent clinical and neuropsychological assessment at the time of scan acquisition. Neuropsychological assessments included: The Clinical Dementia Rating sum of boxes (CDR-sob), Mini-Mental Status Exam, Auditory Verbal Learning Test and AD Assessment Schedule – Cognition. A composite memory score encompassing the Auditory Verbal Learning Test, AD Assessment Schedule – Cognition, Mini-Mental Status Exam and Logical Memory assessments was also utilised[28]. Additionally, a composite executive function score comprising Category Fluency – animals, Category Fluency – vegetables, Trails A and B, Digit span backwards, Wechsler Adult Intelligence Scale (WAIS-R) Digit Symbol Substitution, Number Cancellation and five Clock Drawing items was used[29]. These composite scores were used in formal analyses to represent memory and executive function among subjects. Out of the cognitive tests that were available for ADNI, only Logical Memory – Immediate Recall, Logical Memory – Delayed Recall, Mini-Mental Status Exam and Global CDR scores were available for AIBL, although the same protocols were used. An executive function composite factor was available for AIBL, although it was comprised of CDR-sob, the Stroop test, the FAS test and Category Switch Total[30].

MRI acquisition and pre-processing

MRI scans were available for both ADNI and AIBL. T1-weighted MRI scans were acquired within 10–14 d of the screening visit following a back-to-back 3D magnetisation prepared rapid gradient echo scanning protocol described elsewhere[31]. Images were pre-processed using techniques previously described[32]. Briefly, the SPM12 'New Segmentation' tool was used to normalise images and extract modulated GM and white matter (WM) volume maps to Montreal Neurological Institute space. Maps were smoothed with an 8 mm Gaussian kernel and then used for voxel-wise analyses.

Fluorodeoxyglucose-positron emission tomography

FDG-positron emission tomography images were available only for ADNI. FDG-positron emission tomography acquisition and pre-processing details have been described previously[31]. Briefly, 185 MBq of [18-153-F]-FDG was injected intravenously. After 30 min, six 5-min frames were acquired. Frames of each baseline image series were co-registered to the first frame and combined into dynamic image sets. Each set was averaged, reoriented to a standard $160 \times 160 \times 96$ voxel spatial matrix of resliced 1·5 mm^3 voxels, normalised for intensity and smoothed with an 8 mm full width at the half maximum (FWHM) kernel. In order to derive the standardised uptake value ratio, pixel intensity was normalised according to the pons since it demonstrates preserved glucose metabolism in AD[33]. Normalisation to the pons removed inter-individual tracer metabolism variability. The Montreal Neurological Institute template space was used to spatially normalise images using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/).

Genomic data processing and quality control

Genomic data were only available from ADNI. Quality control of these data was conducted by analysing Hardy–Weinberg equilibrium accepted data for Mendelian inheritance errors. From the entire data set, SNP were selected for further analyses based on Hardy–Weinberg equilibrium $P > 0{\cdot}00001$, MAF > 0·05 %, call rate 95 %. Samples with >5 % missingness were removed. Sample genotypes were imputed using 1000Genomes data with Shapeit/Impute2 software following the protocol described previously[34]. SNP with call rates <95 % or $R^2 \leq 0{\cdot}3$ were withdrawn, leaving 2 976 223 imputed and genotyped SNP after quality control. Subsequently, we *a priori* examined SNP that comprised the *CUBN*, *MTHFR*, *MTRR* and *TCN2* genes.

Statistical analysis

For voxel-wise analysis, second-level linear mixed models in SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/) tested main effects of vitamin B_{12} on regional GM and WM volume as well as FDG, controlling for age, sex, BMI, baseline diagnosis and *APOE4* status. Significance thresholds were set at $P < 0{\cdot}005$ (uncorrected) and $P < 0{\cdot}05$ (corrected) for voxels and clusters, respectively. Results were considered significant at the cluster level. As described previously[35], in order to reduce type 1 error, we utilised a GM threshold of 0·2 to ensure that voxels with <20 % likelihood of being GM were not analysed. For GM and WM, Monte Carlo simulations in ClusterSim (http://afni.nimh.nih.gov/afni/doc/manual/3dClustSim) were used to estimate that 462 contiguous voxels were needed for such a cluster to occur at $P < 0{\cdot}05$. For FDG voxel-wise analyses, Monte Carlo simulations in ClusterSim were used to estimate that 224 contiguous voxels were needed for such a cluster to occur at $P < 0{\cdot}05$.

All genetic association analyses were conducted using PLINK v1.9 (http://www.cog-genomics.org/plink2). The following genes were analysed through linear associations in White participants of European ancestry with a phenotype of the predicted GM and FDG uptake in maxima from voxel-wise analyses: *CUBN*, *MTHFR*, *MTRR* and *TCN2*. Covariates for PLINK analyses included sex, clinical diagnosis, intracranial volume and *APOE4* status. A Holm–Bonferroni threshold for significance was set of 0·05/4, $P < 0{\cdot}0125$[36].

Non-voxel linear mixed regression was conducted using SPSS 25 (IBM Corp.) to test vitamin B_{12} main effects and interactions

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with baseline diagnosis and *APOE4* status, on cognitive scores and biomarkers. Covariates included age, sex and BMI. Years of education were also added as a covariate in models with cognitive scores. Binomial and multinomial logistic regressions were used to assess the OR of a given participant being diagnosed as MCI or AD *v.* CN, or of diagnosis between CN *v.* MCI *v.* AD.

Results

Data summary

ADNI and AIBL clinical, demographics and biomarker data are separately presented in Table 1. A sub-sample of ADNI participants had FDG data, where sub-sample clinical and demographic data are listed in online Supplementary Table S1. Three outliers of each cohort were removed for having vitamin B_{12} levels 3 standard deviations from the mean. Vitamin B_{12} values ranged from 99 to 1146 pg/ml for ADNI participants and for each diagnostic group as follows: CN (157–1146 pg/ml); MCI (157–1084 pg/ml) and AD (99–1121 pg/ml). Vitamin B_{12} values for AIBL participants ranged from 117 to 1149 pg/ml and for each diagnostic group as such: CN (121–1149 pg/ml); MCI (176–1139 pg/ml) and AD (117–895 pg/ml). The reference range is 200–950 pg/ml[3,37]. In the ADNI sub-sample, eleven participants had values below the reference range and four participants had values above the reference range. In the AIBL sub-sample, sixteen participants had values below the reference range and four participants had values above the reference range.

Baseline diagnosis: differences in vitamin B_{12} levels

Binary and multinomial logistic regression indicated that serum vitamin B_{12} levels did not predict a higher likelihood of being diagnosed as MCI, AD or cognitively impaired (MCI + AD) *v.* the CN reference group.

Vitamin B_{12} and regional grey matter volume

Voxel-wise analyses regressed serum vitamin B_{12} against regional GM at baseline separately for 144 participants from ADNI and 401 participants from AIBL. For ADNI participants,

Table 1. Demographics for Alzheimer's Disease Neuroimaging Initiative (ADNI) and Australian Imaging, Biomarker & Lifestyle Flagship Study of Ageing (AIBL) participants with grey matter (GM) images†
(Mean values and standard deviations; numbers; percentages)

	CN		MCI		AD	
	Mean	SD	Mean	SD	Mean	SD
ADNI						
n	39		73		31	
Age (years)	74·00	5·17	73·16	7·24	73·58	6·19
Serum vitamin B_{12} (pg/ml)	439·67	213·00	382·63	149·22	420·51	238·41
Serum homocysteine (μmol/l)	10·67	2·98	11·59	3·47	11·82	4·46
BMI (kg/m^2)	26·61	4·04	25·42	3·68	25·14	3·90
Sex (% female)	51·3		41·4		61·3	
% *APOE4* carriers***	25·6		61·7		74·2	
AIBL						
n	311		59		31	
Age*** (years)	71·17	6·49	75·37	7·25	73·23	8·31
Serum vitamin B_{12} (pg/ml)	415·55	163·63	429·70	156·91	405·21	181·82
Serum homocysteine (μmol/l)	9·39	3·28	10·41	3·71	10·21	3·15
BMI (kg/m^2)	26·29	3·94	26·31	4·54	25·86	3·89
Sex (% female)**	56·6		35·6		45·2	
% *APOE4* carriers***	29·9		47·5		67·7	

CN, cognitively normal; MCI, mild cognitive impairment; AD, Alzheimer's disease.
**$P < 0·01$, ***$P < 0·001$.
† χ^2 Analyses were conducted to examine differences between sex and *APOE4* status. ANOVA was otherwise used.

Table 2. Regional associations of higher serum vitamin B_{12} and less grey matter volume in the Alzheimer's Disease Neuroimaging Initiative*

Location	*t*	X	Y	Z	Cluster size (voxels)
Precuneus (R)	4·21	10	−54	27	507
Posterior cingulate gyrus (R)	3·17	8	−46	22	
Precuneus (R)	2·70	14	−62	33	
Middle frontal gyrus (R)	3·92	−24	51	6	754
Middle frontal gyrus (L)	3·35	−24	24	39	
Middle frontal gyrus (L)	3·24	−38	48	20	
Middle frontal gyrus (R)	3·85	26	51	3	2453
Inferior frontal gyrus (R)	3·75	44	39	27	
Inferior frontal gyrus (R)	3·62	51	32	8	

R, right hemisphere; L, left hemisphere.
* This table depicts regions where all subjects had less grey matter volume per unit increase in vitamin B_{12}. The highest *t* value for a given cluster of significant, contiguous voxels is shown. For clusters that extended over more than 15 mm, the highest *t* value in those areas is indicated. Coordinates are in Montreal Neurological Institute atlas space. Brains are oriented in neurological space.

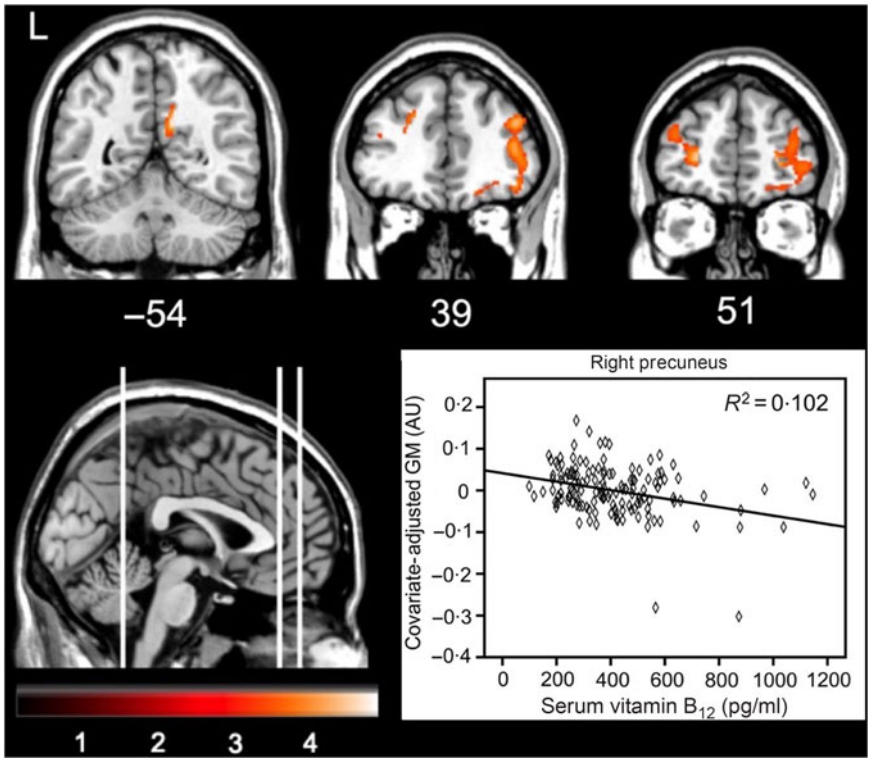

Fig. 1. Brain areas showing less grey matter (GM) corresponding to increased vitamin B_{12} in Alzheimer's Disease Neuroimaging Initiative participants. The graph depicts the relationship at the maximum voxel in the right precuneus. AU, arbitrary units.

higher plasma vitamin B_{12} was correlated with less GM in three clusters (Table 2). The most significant cluster consisted of 507 voxels primarily in the right precuneus and posterior cingulate gyrus (Fig. 1). Other clusters spanned the left middle and inferior frontal gyri. When examining each diagnostic group separately, only the MCI participants showed significant associations between vitamin B_{12} and GM. Specifically, higher vitamin B_{12} in MCI was associated with less GM in the right thalamus, precuneus and calcarine cortex ($k = 1023$).

For AIBL participants, higher serum vitamin B_{12} was correlated with more GM ($k = 559$) in the right amygdala and superior temporal pole. See Table 3 for a full listing of significant clusters. When examining each diagnostic group separately, only the CN participants showed significant associations, with more vitamin B_{12} associated with more GM in three significant clusters: one in the right superior frontal gyrus ($k = 1186$), one in the right precuneus ($k = 2105$) and one in the right supplementary motor area ($k = 496$).

In ADNI where CSF was largely available, we then tested interactions with vitamin B_{12} and binary cut-offs for CSF AD biomarkers[38]. For CSF A$\beta_{1\text{-}42}$, there was a positive interaction ($P < 0{\cdot}05$, family-wise error (FWE) corrected), such that higher vitamin B_{12} was related to more GM volume in adults with *v.* without high amyloid accumulation. One cluster was present in the right middle frontal gyrus ($k = 522$), one in the right supramarginal gyrus ($k = 739$) and left superior frontal gyrus ($k = 464$). This interaction was not significantly associated with less GM. For CSF total tau, there was a negative interaction ($P < 0{\cdot}05$, FWE corrected), such that vitamin B_{12} was related to less GM in aged adults with high tau accumulation. One cluster spanned the left superior frontal gyrus ($k = 604$). This interaction was not significantly associated with more GM.

Vitamin B_{12} and regional white matter volume

Voxel-wise analysis was used to regress plasma vitamin B_{12} against regional WM at baseline for ADNI and AIBL participants, separately. In ADNI, higher vitamin B_{12} was associated with more WM in two small clusters spanning the cerebellum. In AIBL participants, increased vitamin B_{12} was associated with more WM in one small cluster spanning the left insula. For CSF A$\beta_{1\text{-}42}$, there was a negative interaction ($P < 0{\cdot}05$, FWE corrected), such that vitamin B_{12} was related to less WM in individuals with high amyloid accumulation. The significant cluster spanned the cerebellum ($k = 735$). The interaction was not significantly associated with more WM. For CSF total tau, there was a negative interaction ($P < 0{\cdot}05$, FWE corrected), such that vitamin B_{12} was related to less WM in individuals with high tau accumulation. The significant cluster spanned the left cingulum ($k = 2024$). The interaction was not significantly associated with more WM.

Vitamin B_{12} and regional fluorodeoxyglucose metabolism

Voxel-wise analyses regressed plasma vitamin B_{12} concentrations against FDG uptake in 151 ADNI participants. Higher plasma vitamin B_{12} was correlated with less glucose metabolism in the right calcarine and precuneus, where Fig. 2 illustrates the relationship at the maximum voxel in the right calcarine (24, −48, 6). See Table 4 for a full listing of significant clusters. When

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Table 3. Regional associations of higher serum vitamin B_{12} and more grey matter volume in the Australian Imaging, Biomarker & Lifestyle Flagship Study of Ageing*

Location	*t*	X	Y	Z	Cluster size (voxels)
Amygdala (R)	3·57	30	4	−24	559
Superior temporal pole (R)	3·13	45	14	−18	

R, right hemisphere.
* This table depicts regions where all subjects had more predicted grey matter volume per unit increase in vitamin B_{12}. The highest *t* value for a given cluster of significant, contiguous voxels is shown. For clusters that extended over more than 15 mm, the highest *t* value in those areas is indicated. Coordinates are in Montreal Neurological Institute atlas space. Brains are oriented in neurological space.

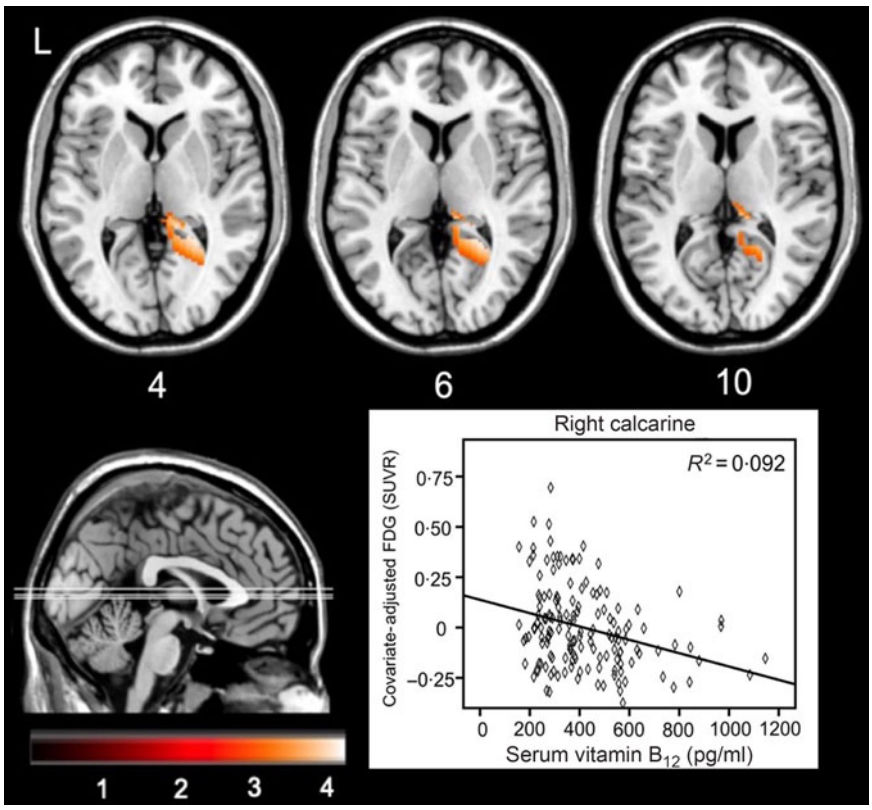

Fig. 2. Brain areas showing less fluorodeoxyglucose (FDG) metabolism corresponding to increased vitamin B_{12}. The graph depicts the relationship at the maximum voxel in right calcarine cortex. SUVR, standardised uptake value ratio.

Table 4. Regional associations of higher serum vitamin B_{12} and less fluorodeoxyglucose (FDG) glucose uptake in the Alzheimer's Disease Neuroimaging Initiative*

Location	*t*	X	Y	Z	Cluster size (voxels)
Calcarine (R)	3·90	24	−48	6	2166
Precuneus (R)	3·67	14	−40	4	
Thalamus (R)	3·40	10	−32	10	

R, right hemisphere.
* This table depicts regions where all subjects had less predicted FDG glucose uptake per unit increase in vitamin B_{12}. The highest *t* value for a given cluster of significant, contiguous voxels is shown. For clusters that extended over more than 15 mm, the highest *t* value in those areas is indicated. Coordinates are in Montreal Neurological Institute atlas space. Brains are oriented in neurological space.

conducting the analyses stratified by baseline diagnosis group, no clusters survived correction for CN and AD participants. For MCI participants, higher vitamin B_{12} was associated with less FDG uptake in one large cluster ($k = 2166$) spanning the right lingual gyrus, precuneus and posterior thalamus mostly in the pulvinar nucleus.

Similar to GM, interactions were then tested with vitamin B_{12} and cut-offs for AD biomarkers. For CSF $A\beta_{1\text{-}42}$, a positive interaction ($P < 0·05$, FWE corrected) indicated that higher vitamin B_{12} was related to more GM in adults with high amyloid accumulation in one cluster spanning mid-cingulate gyrus ($k = 499$). This interaction was not significantly associated with less FDG

Table 5. Association between cubilin SNP and predicted grey matter at the maximal voxel in the right precuneus

Chromosome	SNP	bp	A1	*n*	*β**	*F*	*P*
10	rs10904831	16931344	T	111	−0·08162	−2·542	0·01249
10	rs2356587	16979380	C	111	0·02488	2·594	0·01085
10	rs1801234	16979661	C	111	0·02488	2·594	0·01085
10	rs7072262	17057766	T	109	−0·02607	−2·741	0·007229
10	rs4614335	17059826	A	108	−0·0262	−2·740	0·007252
10	rs12218279	17060676	A	109	−0·02607	−2·741	0·007229
10	rs7897550	17064992	A	111	−0·02482	−2·636	0·009651
10	rs11254331	17065357	A	110	−0·02465	−2·612	0·01035
10	rs17139621	17065761	C	110	−0·02465	−2·612	0·01035

A1, minor allele.
* β Values represent the difference in the predicted value of grey matter with an increase from no risk alleles to one risk allele or two risk alleles.

metabolism. For total tau, by contrast, higher vitamin B_{12} in adults with high tau accumulation was related to less FDG metabolism in the same mid-cingulate cluster ($k = 244$). The interaction was not significantly associated with more FDG metabolism.

Genotype analyses for vitamin B_{12} and predicted differences in grey matter and fluorodeoxyglucose

Next, in ADNI, SNP for genes associated with vitamin B_{12} uptake, transport and metabolism were used as predictors of interest, to see if genotypes might explain the wide variance seen in vitamin B_{12} associations. Linear regression in PLINK tested the additive genetic model of each SNP for main effect associations with GM and FDG predicted values, from the voxel-wise analyses reported above. Nine SNP in the *CUBN* gene were significantly associated with GM and passed Holm–Bonferroni correction, seven of which were detrimental for individuals who had the minor allele and two of which were beneficial (Table 5). One SNP, rs7918972 in the *CUBN* gene, was significantly associated with FDG and passed Holm–Bonferroni correction, which was associated with less FDG uptake ($\beta = -0{\cdot}094$, $P = 0{\cdot}0065$). *MTHFR, MTRR and TCN2* SNP were not significantly associated with GM or FDG vitamin B_{12} predicted values.

For vitamin B_{12} levels and genotype, minor allele counts of the ten significant *CUBN* SNP identified in our GM and FDG analyses were not significantly associated with vitamin B_{12} levels.

Vitamin B_{12} and associations with cognition and biofluid markers

Vitamin B_{12} was not significantly correlated with CDR Global scores, CDR-Sob 11, Mini-Mental Status Exam, composite executive factors, or the composite memory factor for ADNI or AIBL participants. There was a significant baseline diagnosis × vitamin B_{12} interaction for predicting the Auditory Verbal Learning Test learning score for ADNI participants ($P = 0{\cdot}048$). Higher vitamin B_{12} was associated with better scores in AD (β 0·0027 (SE 0·001), $P = 0{\cdot}042$) but trending worse scores in MCI (β −0·0022 (SE 0·001), $P = 0{\cdot}061$).

For biomarkers in plasma, homocysteine was first regressed against vitamin B_{12}. The range of homocysteine values for ADNI participants was 3·9–25·9 μmol/l, by diagnosis: CN (6·1–18·1 μmol/l); MCI (3·9–22·0 μmol/l) and AD (6·1–25·9 μmol/l). The range of homocysteine for AIBL participants was 2·8–35·0 μmol/l, by diagnosis: CN (3·0–35·0 μmol/l); MCI (5·5–26·4 μmol/l) and AD (2·8–18·8 μmol/l). Higher vitamin B_{12} was associated with lower plasma homocysteine levels in ADNI participants (β −0·0034 (SE 0·001), $P = 0{\cdot}001$) and AIBL participants (β −0·0021 (SE 0·001), $P < 0{\cdot}001$). For biomarkers in CSF, there were no significant associations between vitamin B_{12} and CSF total tau, *P*-tau-181 or $A\beta_{1\text{-}42}$ for ADNI participants. Only a very small subset of AIBL participants had available CSF data, so these associations were not assessed.

Discussion

We hypothesised that serum vitamin B_{12} levels may be a useful biomarker for AD-related brain atrophy, hypometabolism and cognitive decline. We originally predicted that there would be an inverse correlation between higher serum vitamin B_{12} levels and lower levels of AD markers, due to previous work from other groups[39,40]. This hypothesis was true for AIBL participants, where higher vitamin B_{12} was related to more GM in the right amygdala and superior temporal pole, which has been shown to progressively decrease in volume across the AD spectrum[41]. This leads us to the hypothesis that vitamin B_{12} is protective against GM deterioration in the cognitively unimpaired population, as the majority of AIBL participants were cognitively unimpaired. Conversely, in ADNI participants, higher serum vitamin B_{12} was instead related to less GM volume and FDG metabolism in precuneus, as well as middle and inferior frontal gyri, where these regions show atrophy, hypometabolism and less default mode neural network strength in aged adults with MCI or AD[42–44]. These results contrast with our AIBL findings and lead us to our second hypothesis that vitamin B_{12} correlates with worse neurological outcomes in the cognitively impaired population, as the majority of the ADNI participants in this cohort were cognitively impaired.

In support of our first hypothesis and the positive correlation between vitamin B_{12} and neurological outcomes among AIBL participants, factor analysis of dietary patterns among a group of cognitively unimpaired adults showed that diets that were especially rich in vitamin D, vitamin B_{12} and Zn were associated with increased GM volume in the temporal and frontal cortices[22]. Additionally, Erickson *et al.* conducted a study with

3-d food recalls and cross-sectional MRI scans among thirty-two cognitively unimpaired older adults[45]. The group found that the individuals with higher vitamin B_{12} intake had increased GM in both the left and right superior parietal sulcus. Lastly, in patients with their first lacunar stroke, lower serum vitamin B_{12} was correlated with more severe Fazekas scale graded periventricular WM lesions[46].

Supplementation with vitamin B_{12} in individuals with dementia has been inconclusive. For example, there was no significant improvement in memory or cognition in individuals with dementia who had low levels of vitamin B_{12} and were subsequently supplemented; however, the supplemented individuals showed better verbal fluency scores compared with non-supplemented controls[47]. However, the Homocysteine and B Vitamins in Cognitive Impairment (VITACOG) trial showed that B-vitamin supplementation was related to positive cognitive outcomes in individuals in the top tertile of baseline *n*-3 fatty acids, compared with individuals with lower baseline *n*-3 fatty acid levels, perhaps due to a synergistic effect on phospholipid production for the brain[48].

For further explanation of our second hypothesis, vitamin B_{12} levels in the context of other disease states and AD may support it being an indicator of pathological load later in a given disease process. For example, plasma vitamin B_{12} levels were correlated with increased all-cause mortality in women aged 85 years or greater[49]. In addition, Arendt *et al.* found that in a population of Danish patients who had serum vitamin B_{12} tested and recorded in their electronic medical record, patients with higher vitamin B_{12} had a higher overall cancer risk within 1 year of follow-up[50]. The mechanism behind this relationship was not known; however, the authors postulated that vitamin B_{12} may rise along with malignant processes[50]. Also, individuals with hyperlipidaemia and non-insulin-dependent diabetes had significantly higher levels of vitamin B_{12} compared with healthy controls[51]. Additionally, higher serum vitamin B_{12} may be a sign of its decreased cellular uptake[52]. Vascular damage is a common feature of AD[53], and the vascular endothelium via the CD320 receptor may mediate the homoeostasis between the serum and tissue homoeostasis of vitamin B_{12}[54]. Finally, we found that higher serum vitamin B_{12} was related to better memory performance and more GM in AIBL and seemed to be driven by CN participants, which make up a majority of the AIBL cohort. By contrast, higher vitamin B_{12} was associated with worse memory performance, less GM and less FDG in MCI participants, which constitute the majority of the ADNI1 cohort. These differing patterns of association may be due to MCI *v.* CN participants usually having more tau accumulation[55].

As an alternative or concurrent explanation for our ADNI findings, it has also been shown that vegetarians, though unlikely to be classified as clinically deficient, are more likely to have low-normal levels of vitamin B_{12}[56]. This could likely be extended to individuals who consume plants as a larger portion of their meals, compared with meat. Perhaps, vitamin B_{12} status may act as an indicator of the ratio of meat intake:plant intake in the diet, and this may manifest in predicting AD outcomes, which have also been linked to plant-based dietary habits[57]. It is still puzzling that we found a negative relationship between vitamin B_{12} and neural outcomes in light of the inverse correlation between serum vitamin B_{12} and homocysteine levels. Hooshmand *et al.* found that among 2570 individuals 60 years and older, higher serum methionine:serum total homocysteine ratios were associated with lower risk for dementia and AD and higher serum vitamin B_{12} was positively correlated with methionine:homocysteine ratios. The vast majority of patients in this study were free of dementia or AD[58]. Potentially in our ADNI cohort, vitamin B_{12} still is taken up by the liver in individuals with higher serum vitamin B_{12} to reduce homocysteine production from methionine, but their high vitamin B_{12} levels may be indicative of other tissues, such as the brain, being unable to transport vitamin B_{12} or utilise it properly.

Alternatively, genetic polymorphisms in B vitamin uptake, transport and metabolism may modify how vitamin B_{12} is utilised and affect vitamin B_{12} levels themselves. We found that minor allele polymorphisms in the *CUBN* gene tracked the association between high vitamin B_{12} and less regional GM. Besides its role in receptor-mediated uptake of vitamin B_{12} in the ileum, CUBN is also an apo receptor and is involved in the absorption of high-density lipoproteins in the kidney[59]. Perhaps, *CUBN* polymorphisms, some of which we have shown are associated with higher serum vitamin B_{12} levels, may lead to decreased apo reuptake in the kidneys, which may increase dementia risk.

There are several limitations of this secondary data analysis study. First, only vitamin B_{12} was measured in the participants. Ideally, holotranscobalamin and methylmalonic acid would also have been measured, which would indicate the level of vitamin B_{12} available for cellular uptake, and the absence of vitamin B_{12} from necessary methylation reactions, respectively[60]. Current practice in the healthcare field is to test serum vitamin B_{12} levels or holotranscobalamin first (if a patient has risk factors for vitamin B_{12} deficiency); a serum vitamin B_{12} level of <200 pg/ml is considered deficient[3]. Subsequently, current practice is for clinicians to test an additional metabolic indicator, either methylmalonic acid or homocysteine[61]. Second, it is difficult to determine the mechanisms involved that affect an individual's vitamin B_{12} levels. This can be impacted by proton pump inhibitors[62], level of animal product intake[63] and genetic variability[25] as we have illustrated. Perhaps, the interaction between vitamin B_{12} and one or more of these variables may play a role in cognitive decline. We were also unable to assess associations with physical activity, circulating vitamin B_{12}, and neurological outcomes, as these data were not collected in ADNI or available to us through AIBL. Additionally, FDG scans, genetic and CSF biomarker data were not available in AIBL, which limited our ability to compare ADNI and AIBL. Lastly, because the overwhelming majority of ADNI participants are of European ancestry, we were only able to reliably test the interactions between genetic data and volumetric/function brain outcomes in those individuals. It would be worthwhile to determine if similar genes are implicated in an African American cohort, as the incidence of AD is much higher among African Americans compared with Whites of European descent[64].

Conclusion

This study showed contrasting associations between vitamin B_{12} and neurological outcomes among an Australian cohort with a

strong cognitively unimpaired makeup and a North American cohort made up mostly of participants with some degree of cognitive impairment. While we found that vitamin B_{12} was associated with positive structural associations in the brain in the Australian cohort which was mostly cognitively unimpaired, we found that vitamin B_{12} was correlated with detrimental outcomes among the North American cohort, which consisted mainly of participants with some degree of cognitive impairment. This lends to our hypothesis that vitamin B_{12} may be protective of neurological decline in the healthy population, but may increase for an undetermined reason in those that are experiencing cognitive decline. Additionally, we have shown that these results may be influenced by genetic mutations related to vitamin B_{12} uptake, as well as CSF markers of amyloid and total tau accumulation that may reflect or work in concert with baseline clinical diagnosis. Future research should focus on the rate of uptake of vitamin B_{12} into healthy and diseased neuronal cells, the role of other B vitamin markers in AD onset and progression, and determine if high amyloid or tau accumulation affects the function or efficacy of vitamin B_{12}.

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K. E. M. and A. A. W. formulated the research question and design and carried out the study. K. E. M., A. A. W. and A. D. C. M. analysed the data. K. E. M. and A. A. W. wrote the manuscript in consultation with J. P. M. and K. A. All authors provided critical feedback.

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Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114520000951

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