Supplementary Material

B Vitamins Prevent Iron-Associated Brain Atrophy and Domain-Specific Effects of Iron, Copper, Aluminum, and Silicon on Cognition in Mild Cognitive Impairment

Supplementary Table 1. Baseline and end of study serum concentrations of metals, silicon, Cys, and tHcy.

Variable (n = 97-133)	Mean ± SD at baseline		Mean ± SD at end-of-study (EOS)		*p* B-vitamin versus placebo[#]		*p* EOS versus baseline[#]	
	Placebo group	B-vitamin group	Placebo group	B-vitamin group	Baseline	EOS	Placebo group	B-vitamin group
Fe, mg/L	3.64±2.70	3.13±2.18	0.98±0.32	0.99±0.25	0.262	0.935	**4.E-26**	**2.E-22**
Cu, mg/L	0.96±0.19	1.00±0.22	1.06±0.21	1.05±0.18	0.181	0.886	**2.E-8**	**1.E-4**
Al, µg/L	81±54	80±104	38±23	40±28	0.944	0.543	**2.E-9**	**1.E-5**
Si, mg/L	5.4±2.3	5.2±2.2	7.0±1.9	6.9±2.0	0.520	0.508	**9.E-8**	**2.E-7**
As, µg/L	9.0±21.0	5.5±9.4	1.1±1.6	1.5±2.8	0.130	0.214	**2.E-10**	**5.E-7**
Cys, µM	336.5±44.4	323.9±43.3	342.8±50.0	327.2±39.8	**0.018**	**0.010**	0.171	0.229
tHcy, µM	12.1±4.1	11.8±3.4	13.1±4.7	8.9±2.2	0.503	**0.000**	**0.112**	**0.000**
[#]*p* values were derived from Log-transformed data.								

Supplementary Table 2. Determinants of serum iron (Fe), silicon (Si), copper (Cu), aluminum (Al), and arsenic (As) at baseline.

Variable (n=192)	**LnFe_1**				**LnSi_1**				**LnCu_1**				**LnAl_1**				**LnAs_1**			
	Pearson correlation[#]		Multiple regression*		Pearson correlation		Multiple regression*		Pearson correlation		Multiple regression*		Pearson correlation		Multiple regression*		Pearson correlation		Multiple regression*	
	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
LnFe_1					0.59	**0.000**	0.56	**0.000**					0.27	**0.000**					0.28	**0.003**
LnSi_1	0.59	**0.000**	0.57	**0.000**					0.13	**0.040**	0.16	**0.019**	0.33	**0.000**	0.28	**0.000**	-0.28	**0.000**	-0.43	**0.000**
LnCu_1					0.13	**0.040**	0.14	**0.010**					0.11	0.061						
LnAl_1	0.27	**0.000**			0.33	**0.000**	0.15	**0.006**	0.11	0.061										
LnAs_1			0.17	**0.003**	-0.28	**0.000**	-0.27	**0.000**												
LntHcy_1	0.19	**0.004**			0.24	**0.000**														
LnCystathionine_1	-0.15	**0.020**	-0.20	**0.000**			0.15	**0.007**									0.12	**0.049**	0.12	**0.089**
LnCys_1											-0.11	**0.092**					0.19	**0.004**	0.18	**0.009**
LnTaurine_1	-0.24	**0.000**	-0.18	**0.001**					0.33	**0.000**	0.28	**0.001**					0.10	0.088		
LnCreatinine_1	0.14	**0.025**			0.10	0.095			-0.15	**0.020**										
LnPhAcase	-0.32	**0.000**	-0.21	**0.000**	-0.20	**0.003**							-0.22	**0.002**	-0.16	**0.026**				
Age	0.15	**0.021**			0.11	0.060											0.09	0.097		
Sex									0.36	**0.000**	0.29	**0.000**								
			F = 32.2, *p* = 0.000, Adjusted R^2 = 0.46				F = 33.8, *p* = 0.000, Adjusted R^2 = 0.46				F = 13.3, *p* = 0.000, Adjusted R^2 = 0.21				F = 12.8, *p* = 0.000, Adjusted R^2 = 0.11				F = 8.8, *p* = 0.000, Adjusted R^2 = 0.14	
	[#] One-sided T-test. Empty fields indicate no correlation. Ln, natural logarithm; _1, baseline; tHcy, total homocysteine; Cys, cysteine; PhAcase, phenylacetate hydrolase (PON1). *Variables included in a multiple regression model are indicated by numerical entries. Empty fields indicate variables that were non-significant and not included in a model.																			

Supplementary Table 3. Association of cognition with metals, silicon, and brain volume at baseline – Pearson correlation*

Variable (n=182-192)	Semantic memory				Verbal episodic memory				Attention/processing speed								Executive function		Global cognition	
	LnCategory_Fluency_1		LnGraded_Naming_1		LnHVLT-TR_1		LnHVLT-DR_1		LnTrail_Making_A_1		LnTrail_Making_B_1		LnMap_Search_1		LnSDMT_1		LnCLOX_1		LnMMSE_1	
	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
LnFe_1	-0.24	**0.000**	-0.12	**0.043**	-0.17	**0.011**							-0.17	**0.010**						
LnSi	-0.18	**0.006**	-0.13	**0.038**	-0.15	**0.018**			0.15	**0.019**	0.13	**0.039**	-0.19	**0.004**	-0.14	**0.025**	0.20	**0.007**	**-0.13**	**0.041**
LnAl_1									-0.12	**0.046**							-0.13	0.056		
LnCu_1					0.11	0.071	0.15	**0.024**												
LntHcy_1	-0.26	**0.000**	-0.18	**0.002**	-0.15	**0.016**	-0.12	0.052	0.10	**0.050**	0.21	**0.000**	-0.22	**0.000**	-0.21	**0.001**	0.23	**0.001**		
LnCys_1	-0.11	**0.041**	-0.13	**0.020**							0.13	**0.020**			-0.22	**0.000**				
LnBrain_Volume_1	0.14	**0.036**	0.17	**0.012**	0.14	**0.032**	0.15	**0.037**	-0.22	**0.001**	-0.27	**0.000**	0.28	**0.000**	0.39	**0.000**			0.017	**0.012**
Age	-0.24	**0.000**	-0.22	**0.000**	-0.18	**0.000**	-0.15	**0.009**	0.27	**0.000**	0.33	**0.000**	-0.30	**0.000**	-0.34	**0.000**	0.13	**0.033**	-0.11	**0.042**
Sex (1, male; 0, female)	0.31	**0.000**	-0.14	**0.009**	0.25	**0.000**	0.20	**0.001**			0.09	0.069								

*Ln, natural logarithm; _1, baseline; One-sided T-test. Empty β and *P* cells indicate no correlation.

Note: Higher scores indicate better performance in all tests except the Trail_Making_A and _B, where longer time taken indicates poorer performance and the CLOX test, where higher indicates worse performance.

Supplementary Table 4. Association of cognition with metals and silicon at baseline – multiple regression analysis*.

Variable (n = 182-192)	Semantic memory		Verbal episodic memory				Attention/processing speed						Executive function	
	LnCategory_Fluency_1		LnHVLT-TR_1		LnHVLT-DR_1		LnTrail_Making_A_1		LnMap_search_1		LnSDMT_1		LnCLOX_1‡	
	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
LnFe_1	-0.16 -0.21	**0.045** **0.003**†	-0.17	**0.040**^					-0.16 -0.15	**0.052**^ **0.044**†				
LnSi_1			-0.20	**0.019**			0.25	**0.005**	-0.20	**0.022** **0.010**†	-0.16	**0.034**†	0.20	**0.015**†
LnAl_1	0.12 0.15	**0.119** **0.032**†	0.19 0.16	**0.029** **0.059**^			-0.26	**0.004**	0.09 0.16	0.161 0.291^	0.15	**0.046**†	-0.18	**0.028**†
LnCu_1					0.19	**0.036**								
LntHcy_1	-0.14 -0.14	**0.093** **0.052**†											0.18	**0.037**†
LnCys_1		NS		NS		NS		NS		NS		NS		NS
LnBrain_Volume_1	0.15	**0.078**	0.17	**0.056**	0.27	**0.004**	**-0.11**	**0.209**	0.32	**0.000**				
Age	-0.16	**0.070**		**NS**		**NS**	**0.14**	**0.045**		**NS**	**-0.29**	**0.000**		**NS**
Sex	0.29	**0.000**	**0.22**	**0.006**		**NS**		**NS**		**NS**		**NS**		**NS**
	F = 6.8, *p* = 0.000, Adjusted R^2 = 0.19		F = 5.1, *p* = 0.001, Adjusted R^2 = 0.12		F = 5.1, *p* = 0.000, Adjusted R^2 = 0.11		F = 3.9, *p* = 0.000, Adjusted R^2 = 0.09		F = 5.7, *p* = 0.000, Adjusted R^2 = 0.14		F = 6.4, *p* = 0.000, Adjusted R^2 = 0.10		F = 3.6, *p* = 0.004, Adjusted R^2 = 0.08†	
	*Ln, natural logarithm; _1, baseline; NS, not significant. Adjusted for LnBrain_volume_1, age, and sex. † Model without Brain_Volume_1. ^ Model without Si. ‡ Only tHcy remained significant in a model with Brain_Volume_1. ‡ Model with Si and without Fe. Note: Trail_Making_A_1 score indicates worse cognitive performance.													

Supplementary Table 5. Determinants of cognition at the end of study - B vitamin group*.

Variable	Multiple regression#											
	Global Cognition		Semantic Memory				Attention/speed					
	LnMMSE_2		LnCategory_Fluency_2		LnGraded_Naming_2		LnTrail_Making_A_2		LnTrail_Making_B_2		LnMap_Search_2	
	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
LnHcy_1	-0.06	0.630	0.13	0.138		NS		NS		NS		NS
LnFe_1	0.19	0.165	0.07	0.445	0.06	0.601	0.01	0.925	0.12	0.369	-0.04	0.790
LnSi_1			-0.27	**0.006**	-0.08	0.473	-0.09	0.563	0.23	0.092	-0.15	0.299
LnAl_1	-0.02	0.864					0.17	0.264	-0.18	0.205	0.15	0.343
LnCu_1							-0.02	0.912	0.21	0.094	-0.17	0.205
LnAtrophy_rate	-0.32	**0.008**	-0.11	0.174	-0.14	0.182	0.18	0.172	-0.02	0.873	-0.04	0.753
LnMMSE_1	0.46	**0.001**										
LnCategory_Fluency_1			0.69	**0.000**								
LnGraded_Naming_1					0.67	**0.000**						
LnTrail_Making A_1							0.59	**0.000**				
LnTrail_Making B_1									0.52	**0.000**		
LnMap Search_1											0.51	**0.000**
	F = 3.3, p = 0.003, Adjusted R^2 = 0.28		F = 13.2, p = 0.000, Adjusted R^2 = 0.61		F = 6.7, p = 0.000, Adjusted R^2 = 0.52		F = 2.9, p = 0.000, Adjusted R^2 = 0.27		F = 3.9, p = 0.000, Adjusted R^2 = 0.27		F = 3.6, p = 0.000, Adjusted R^2 =0.34	
	* Ln, natural logarithm; _1, baseline; _2, end of study; NS, not significant. # Models included other variables that were tested in the placebo group. Note: higher Trail_Making_A_2 and Trail_Making_b_2 scores indicate worse cognitive performance.											