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Association of Cognitive Impairment with Combinations of Vitamin B_{12}–Related Parameters

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BACKGROUND: Low vitamin B_{12} concentrations have been associated with higher risks of cognitive impairment, but whether these associations are causal is uncertain. The associations of cognitive impairment with combinations of vitamin B_{12}, holotranscobalamin, methylmalonic acid, and total homocysteine, and with the vitamin B_{12} transport proteins transcobalamin and haptocorrin, have not been previously studied.

METHODS: We performed a population-based cross-sectional study of 839 people 75 years old or older. We examined the association of cognitive function as measured by mini–mental state examination scores, with markers of vitamin B_{12} status. Spearman correlations as well as multivariate-adjusted odds ratios and 95% CIs for cognitive impairment were calculated for extreme thirds of serum concentrations of vitamin B_{12}, holotranscobalamin, methylmalonic acid, total homocysteine, combination of these markers in a wellness score, heaptocorrin, and transcobalamin for all data and with B_{12} analogs in a nested case-control study.

RESULTS: Cognitive impairment was significantly associated with low vitamin B_{12} [odds ratio 2.3 (95% CI 1.2–4.5)]; low holotranscobalamin [4.1 (2.0–8.7)], high methylmalonic acid [3.5 (1.8–7.1)], high homocysteine [4.8 (2.3–10.0)] and low wellness score [5.1 (2.61–10.46)]. After correction for relevant covariates, cognitive impairment remained significantly associated with high homocysteine [4.85 (2.24–10.53)] and with a low wellness score [5.60 (2.61–12.01)] but not with transcobalamin, haptocorrin, or analogs on haptocorrin.

CONCLUSIONS: Cognitive impairment was associated with the combined effects of the 4 biomarkers of vitamin B_{12} deficiency when included in a wellness score but was not associated with binding proteins or analogs on haptocorrin.

Low vitamin B_{12} (B_{12})[5] concentrations have been associated with higher risks of cognitive impairment and dementia, and with more rapid rates of cognitive decline, but it is unclear if these associations are causal *(1–3)*. Randomized trials of dietary supplementation with B_{12} have not demonstrated any beneficial effects on cognitive function *(4–8)*. The discrepant results of the observational studies and the randomized trials suggest a need for more detailed analyses of the associations of other markers of B_{12} status with cognitive impairment.

Two different proteins, transcobalamin (TC) and haptocorrin (HC), bind and transport B_{12} in the blood *(9)*. Only the fraction of B_{12} bound to TC (holoTC) can be taken up by all cells via the TC receptor *(9–11)*. Inside the cells, B_{12} acts as a coenzyme for 2 different enzymes, methylmalonyl-CoA mutase and methionine synthase *(12)*. Insufficient B_{12} in the cells, therefore, causes an increase in the concentrations of the metabolites, methylmalonic acid (MMA) and total homocysteine (tHcy). The biological function of circulating HC is uncertain, but its ability to bind the so-called B_{12} analogs (corrinoids differing from active forms of B_{12}) suggests that it might protect cells from possible toxicity of these analogs *(13)*.

The discrepant results of the associations of cognitive impairment with B_{12}, holoTC, MMA, and tHcy may reflect their limitations as markers to accurately reflect intracellular B_{12} status. We recently introduced a model in which we took all 4 markers of B_{12} status into account and expressed the combined results either as a distribution surface [$x = (\text{holoTC} \times B_{12})^{1/2}$; $y =$

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[5] Nonstandard abbreviations: B_{12}, vitamin B_{12}; TC, transcobalamin; HC, haptocorrin; holo, saturated with B_{12} or analogues; MMA, methylmalonic acid; tHcy, total homocysteine; MMSE, mini–mental state examination; TCsat, TC saturation; corHC, corrinoids on HC; aHC, analogues on HC; OR, odds ratio.

$0.5 \times \log(\text{MMA} \times \text{tHcy})$; z = the number of participants] or as a "wellness score" *(14)*.

To help elucidate the relevance of overall B_{12} status for cognitive impairment, we assessed the association of cognitive impairment with B_{12}, holoTC, MMA, and tHcy, and compared that to the combined use of the 4 markers expressed either by the distribution surface or by a wellness score. We further examined the association of cognitive impairment with serum concentrations of the B_{12}-binding proteins TC and HC, as well as with B_{12} or analogs attached to HC.

Participants and Methods

PARTICIPANTS AND SAMPLE COLLECTION

The study population for this population-based cross-sectional study was made up of community-dwelling people living in Banbury, Oxfordshire, England *(1)*. Participants (n = 976) were recruited from a random sample of people aged 75 years or older between 2003 and 2004. Individuals underwent a structured interview to record medical history, had blood samples collected, and underwent assessment of cognitive function by use of the mini–mental state examination (MMSE), which resulted in scores ranging from 0 to 30. Cognitive impairment was defined by an MMSE score <22 *(15)*. The study protocol was approved by the Central Oxford Research Ethics Committee (COREC CO2.219).

Venous blood samples were collected and kept chilled until serum was separated at the local hospital laboratory within 2 h of blood collection. Serum was stored at −40 °C to −20 °C for subsequent measurement of B_{12}-related parameters.

LABORATORY METHODS

Vitamin B_{12} was measured by using the ACS Centaur system (Bayer A/S); and holoTC was measured by using an RIA (AXIS-Shield ASA). MMA was measured by gas chromatography–mass spectrometry, and tHcy was measured by using an Abbott IMx autoanalyzer (FPIA), as previously reported *(1)*.

For this study, total TC (reference interval 500–1500 pmol/L) and total HC (reference interval 250–840 pmol/L) were analyzed by use of ELISA *(16–18)*. As previously described, all analyses had an analytical imprecision <7%. TC saturation (TCsat) was calculated as: 100 × holoTC/totalTC (reference interval 2%–17%). Total corrinoids on HC (corHC) (reference interval 210–740 pmol/L) and B_{12} on HC (B_{12}-HC) (reference interval 60–400 pmol/L) were measured simultaneously with an analytical imprecision <13% *(19)*. The total amount of B_{12} analogs on HC (aHC) (reference interval 100–380 pmol/L) was calculated as the difference between corHC and B_{12}-HC. On the basis of the control samples included in each analysis, we determined that all of the components studied were stable for at least 6 years in samples stored at −18 °C.

STATISTICAL AND MATHEMATICAL ANALYSIS

Individuals who had incomplete laboratory data (n = 75) or who reported the use of B_{12} injections or any B-vitamin supplements (n = 48) were excluded. After removal of outliers (total n = 14) with holoTC >240 pmol/L (n = 2), totalTC >2000 pmol/L (n = 8), totalHC >2000 pmol/L (n = 2), or totalHC <200pmol/L (n = 2), 839 individuals were included for assessment of totalTC, TCsat, and totalHC as well as for calculations combining the 4 parameters B_{12}, holoTC, MMA, and tHcy in a wellness score. Overall, the current analyses involved 86% of the total cohort. MMSE scores [median (25%–75% range)] for those excluded [27 (24–28)] did not differ from scores of those included [27 (24–28)]. For the study of corrinoids on HC, a case-control analysis was conducted. It consisted of 80 individuals with MMSE <22, and 83 age- and sex-matched controls with MMSE >28.

The variables of 3-dimensional analysis [$x = (B_{12} \times \text{holoTC})^{1/2}$ and $y = 0.5 \times \log(\text{MMA} \times \text{tHcy})$] were calculated for each individual on the basis of the previously presented model *(14)*. These values were plotted as a 3-dimensional polygon according to their frequency on the (x,y) surface. The sum of 4 gaussian functions was used to approximate the polygon surface and quantify the local peaks of frequency and their distribution. The number of individuals associated with each particular peak was calculated by integration of the corresponding gaussian functions, assuming either completely independent shapes or similar proportions (i.e., mean ratios of height and width).

In addition, we calculated a wellness score for each participant ["wellness parameter" according to the original terminology in the report by Fedosov *(14)*]. The wellness score was a combined expression of the normalized values of B_{12}, holoTC, MMA, and tHcy *(14)*. This expression was based on the logarithmic presentation of the geometric mean of these 4 normalized markers. For each participant, the markers were expressed relative to mean values obtained for the group of reference individuals displaying the largest peak on the distribution surface *(14)*. The wellness score of an individual was calculated as: wellness score = $\log10(\text{holoTC}_{t/n}) + \log10(B_{12t/n}) - \log10(\text{MMA}_{t/n}) - \log10(\text{Hcy}_{t/n})$, where, for example, $\text{MMA}_{t/n} = \text{MMA}_{\text{testperson}}/\text{MMA}_{\text{normal}}$. The value of $\text{MMA}_{\text{testperson}}$ corresponded to MMA of the tested individual, whereas $\text{MMA}_{\text{normal}}$ was the mean "nor-

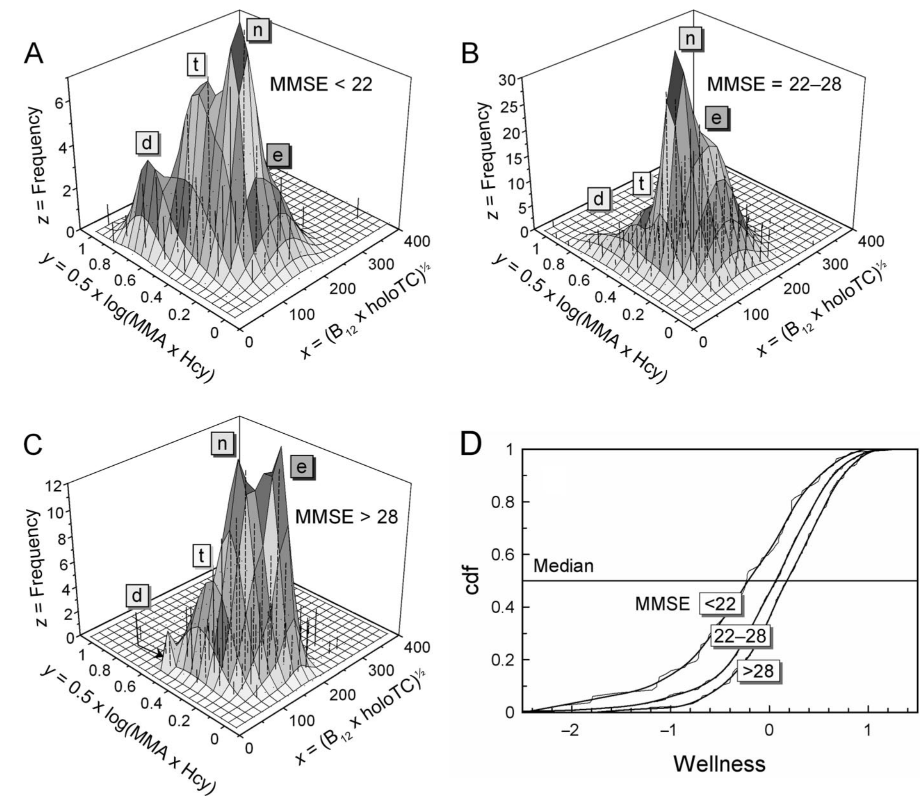

Fig. 1. Combined markers of B_{12} status show a clear redistribution of frequency in favor of poor B_{12} status at decreased cognitive characteristics.

The figure shows the frequency distributions for the population classified by 3 categories according to their MMSE score: (A), MMSE <22 (n = 82); (B), MMSE 22–28 (n = 549); and (C), MMSE >28 (n = 208). A pairwise combination of 4 common markers of B_{12} deficiency (B_{12}, holoTC, MMA, and tHcy) was used to produce the 3-dimensional distributions as previously described *(14)*. The obtained surfaces deviate from normal distribution, and each 1 contains at least 4 overlapping peaks quantified by regression fitting. The peaks are classified according to B_{12} status as deficient (d), transient (t), normal (n), or excellent (e). Note that the frequency axes are not the same in each plot owing to different numbers of participants in each subgroup. The percentage of individuals within each peak was calculated by integration: (A), d = 9.2%, t = 48.5%, n = 25.7%, e = 16.7%; (B), d = 1.4%, t = 8.8%, n = 42.2%, e = 47.6%; (C), d = 0.3%, t = 7.5%, n = 48.8%, e = 43.5%. (D), The cumulative distribution function (cdf) of wellness scores in the 3 MMSE-based subgroups from panels (A), (B), and (C). See Methods for calculation of the wellness score.

mal" value taken from Table 3, subgroup "normal," in the report by Fedosov *(14)*.

The fitting program KyPlot 5 (KyensLab) was used for the above purposes.

The measured variables were summarized as median (range) as well as mean (SD). Spearman correlations between the analyzed parameters were calculated. Logistic regression was used to assess associations of cognitive impairment (MMSE <22), with thirds of serum levels of markers of B_{12} status after adjustment for age and sex or for age, sex, diabetes, cardiovascular diseases, depression, smoking, and treatment with H2 antagonists.

Results

COGNITIVE IMPAIRMENT IS STRONGLY ASSOCIATED WITH COMBINED MARKERS OF B_{12} DEFICIENCY

The associations of cognitive impairment with combinations of B_{12}, holoTC, MMA, and tHcy are presented in Fig. 1. This figure shows the frequency of cases (*z*) plotted vs 2 combined variables: B_{12}-related markers (*x*) and metabolites (*y*) (Fig. 1, A–C). Consistent with previous results *(14)* each peak is classified according to B_{12} status as excellent (e), normal (n), transient (t), or deficient (d). Fig. 1, A–C display the model applied to 3 subgroups of the population, MMSE <22 (n =

Table 1. Characteristics of the total sample set and of the case group (MMSE <22) together with the age- and sex-matched control group (MMSE >28).[a]

		Case vs control (*n* = 163); male (*n* = 36)		
	All data (N = 839); male (*n* = 326), median (range)	Case (*n* = 80), median (range)	Control (*n* = 83), median (range)	*P* value of median test[b]
Age, y	80 (75 to 97)	83 (75 to 97)	82 (76 to 94)	NS
MMSE	27 (6 to 30)	19 (6 to 21)	29 (29 to 30)	***
B_{12}, pmol/L	230 (62 to 600)	200 (85 to 600)	270 (140 to 480)	**
holoTC, pmol/L	71 (4.2 to 230)	64 (10 to 230)	81 (22 to 180)	*
MMA, μmol/L	0.27 (0.13 to 2.6)	0.38 (0.16 to 1.6)	0.25 (0.13 to 0.16)	***
tHcy, μmol/L	13.8 (6.78 to 135)	15.6 (8.49 to 135)	12.8 (8.08 to 27.0)	***
Wellness[c]	0.08 (−3.0 to 1.4)	−0.22 (−2.3 to 11.3)	0.26 (−1.2 to 0.94)	***
totalTC, pmol/L	1000 (380 to 1985)	1020 (64 to 1970)	970 (380 to 199)	NS
TCsat, %	7.2 (0.44 to 29)	6.3 (0.89 to 24)	8.4 (2.6 to 21)	**
totalHC, pmol/L	460 (210 to 1120)	450 (220 to 960)	470 (250 to 930)	NS
corHC, pmol/L		390 (210 to 750)	400 (250 to 750)	NS
B12-HC, pmol/L		180 (90 to 400)	210 (110 to 410)	*
aHC, pmol/L		200 (98 to 440)	190 (97 to 360)	NS
B12-HC/aHC		0.87 (0.45 to 3.1)	1.07 (0.47 to 2.3)	*

[a] For further data, including mean (SD), see online Supplemental Table 1.
[b] The *P* value of the median test comparing differences in medians between the case and control groups for each parameter. ***$P < 0.0001$; **$P < 0.001$; *$P < 0.05$; NS, medians are not significantly different.
[c] The wellness score combines B12, holoTC, MMA, and tHcy, see Participants and Methods for details.

82), MMSE 22–28 (n = 549), and MMSE >28 (n = 208). We found the major peaks to occur at the same coordinates for the 3 groups. The distribution of the pooled population (data not shown) was comparable to that of MMSE 22–28. The percentage of individuals within each peak was calculated by integration. Although the subgroup with MMSE <22 had 56% of individuals within the deficient and transient peaks (Fig. 1A), the representation of those B_{12}-insufficient types decreased to 10.2% at MMSE 22–28 (Fig. 1B) and 7.8% at MMSE >28 (Fig. 1C). The mean MMSE values within the peak fractions of the pooled population increased from 22.5 in the deficient area of metabolites to 26.4 around the peak of "excellent" types (data not shown).

Cumulative distribution functions of the wellness scores for the 3 MMSE-based subgroups are shown in Fig. 1D. Among the individuals with MMSE <22, 34% had wellness scores <−0.5, reflecting poor B_{12} status, whereas in the group with MMSE >28, only 10% had wellness scores <−0.5.

DATA CHARACTERISTICS

Table 1 shows selected characteristics of the overall population and of the nested case-control study population with cognitive impairment. As previously described *(1)*, the overall median age was 80 (range 75–97) years, median MMSE was 27 (6–30), and median B_{12} was 230 (62–600) pmol/L. Cases (n = 80) with cognitive impairment had significantly higher median concentrations of tHcy and MMA compared with age- and sex-matched controls. The cases had lower median B_{12} (200 vs 270 pmol/L), lower median holoTC (64 vs 81), lower B_{12}-HC/aHC ratios (0.87 vs 1.07), and lower median wellness scores (−0.22 vs 0.26) compared with controls. No significant differences between cases and controls were found for totalTC, totalHC, total corrinoids on HC (corHC), and analogs on HC (aHC). Further information is given in Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol57/issue10.

CORRELATIONS BETWEEN MARKERS OF B_{12} STATUS

Table 2 shows the Spearman correlations of MMSE with selected markers of B_{12} status. MMSE correlated significantly with all parameters except for totalTC, totalHC, corHC, and aHC. Notably, B_{12} was strongly correlated with holoTC and totalHC but not with aHC or totalTC. TotalTC was correlated with totalHC but not with B_{12}, B_{12}-HC, or aHC. TotalHC was strongly correlated with B_{12}, aHC, and B_{12}-HC,

Table 2. Spearman correlations (P values) between selected parameters.[a]

	MMSE[b]	B_{12}[b]	Wellness[b]	totalTC[b]	holoTC[b]	TCsat[b]	totalHC[b]	B12-HC[c]	aHC[c]	B12-HC/aHC[c]
MMSE[b]		0.094**	0.19***	−0.065[NS]	0.14***	0.16***	−0.017[NS]	0.17*	−0.08[NS]	0.23**
B_{12}[b]	0.094**		0.76***	−0.063[NS]	0.64***	0.65***	0.50***	0.87***	0.063[NS]	0.72***
Wellness[b]	0.19***	0.76***		−0.012***	0.83***	0.81***	0.16***	0.60***	−0.11[NS]	0.63***
totalTC[b]	−0.065[NS]	−0.063[NS]	−0.012***		0.16***	−0.28***	0.18***	−0.12[NS]	0.12[NS]	−0.22**
holoTC[b]	0.14***	0.64***	0.83***	0.16***		0.88***	0.060[NS]	0.44***	−0.21**	0.58***
TCsat[b]	0.16***	0.65***	0.81***	−0.28***	0.88***		−0.020[NS]	0.51***	−0.28***	0.70***
totalHC[b]	−0.017[NS]	0.50***	0.16***	0.18***	0.060[NS]	−0.020[NS]		0.69***	0.56***	0.16*
B12-HCb[c]	0.17*	0.87***	0.60***	−0.12[NS]	0.44***	0.51***	0.69***		0.28***	0.65***
aHC[c]	−0.08[NS]	0.063[NS]	−0.11[NS]	0.12[NS]	−0.21**	−0.28***	0.56***	0.28***		−0.50***
B12-HC/aHC[c]	0.23**	0.72***	0.63***	−0.22**	0.58***	0.70***	0.16*	0.65***	−0.50***	

[a] *P* values of each correlation are indicated as ****P* < 0.0001; ***P* < 0.001; **P* < 0.05; NS, correlation is not significant.
[b] For data in these rows and columns correlations were calculated on all data (n = 839).
[c] For data in these rows and columns correlations were calculated on case/control data (n = 163). For additional data see online Supplemental Table 2.

but not with holoTC. Similar strong correlations of TCsat to aHC, B_{12}-HC, and the ratio between these 2 were noted.

Additional correlations are shown in online Supplemental Table 2, including those with tHcy and MMA, which were both significantly correlated with all measured markers of B_{12} status, except for totalHC, corHC, and aHC.

ASSOCIATIONS WITH COGNITIVE IMPAIRMENT

Table 3 shows the odds ratios (ORs) and 95% CIs of cognitive impairment as defined by MMSE <22 for extreme thirds of markers of B_{12} status after adjustment for age and sex. Compared to individuals with levels in the upper third, those with wellness scores in the lower third had a 5.1-fold higher risk of cognitive impairment. Individuals with lower-third B_{12} and holoTC showed a 2.3- to 4.2-fold increased risk of cognitive impairment, whereas those with MMA and tHcy in the upper third had a 3.5- to 4.9-fold increased risk of cognitive impairment. After additional adjustment for diabetes, cardiovascular diseases, depression, smoking, and treatment with H2-antagonists, a wellness score in the lower third was associated with a 5.6-fold higher risk of cognitive impairment (Table 3).

Individuals in the lower third of B_{12}-HC and B_{12}-HC/aHC had a 2.5- to 3.2-fold higher risk of cognitive impairment. Similarly, lower third TCsat and corHC were associated with a 3-fold higher risk of cognitive impairment. However, no associations were found between the risk of cognitive impairment and serum concentrations of totalTC, totalHC, or aHC.

Discussion

The present study provided information in addition to that previously published regarding associations of cognitive impairment with markers of B_{12} status *(1)* by assessment of associations of cognitive impairment with modeled combinations of 4 biomarkers of B_{12} status and associations of cognitive impairment with totalTC, totalHC, corHC, and aHC. A strength of this study was the relatively large number of individuals studied and the combined exploration of both established markers of B_{12} status (B_{12}, holoTC, MMA, and tHcy) and novel markers of vitamin B_{12} status (total TC, total HC, aHC). A limitation of the study was that only MMSE was used to assess cognitive function.

THE WELLNESS SCORE IS ASSOCIATED WITH COGNITIVE IMPAIRMENT

The wellness score based on the markers B_{12}, holoTC, MMA, and tHcy has been shown to provide a more efficient estimate of the B_{12} status than the values of the individual parameters *(14)*. We have previously documented that a wellness score of −0.5 is a suitable cutoff for classifying individuals as having an impaired B_{12} status *(14)*. In the present study we examined the association of cognitive impairment with the wellness score.

We also used a 3-dimensional surface, on which each participant is represented by a point incorporating all 4 markers (Fig. 1, A–C). The obtained surfaces did not follow a normal distribution, but demonstrated the presence of several local peaks of frequency with reproducible positions, which were closely related to those previously reported *(14)*. Gaussian peaks of frequency were identified by regression fitting and associ-

Table 3. ORs and 95% CIs of cognitive impairment (MMSE <22) of biomarkers related to vitamin B12 status.[a]

Biomarker (total no. of participants)	Tertile	Mean[b]	Cases, n[c]	Controls, n[d]	Cognitive impairment adjusted for age and sex, OR (95% CI)[e]	Cognitive impairment also adjusted for disease and lifestyle factors, OR (95% CI)[f]
B12 (n = 839), pmol/L	I	160	39	59	2.32 (1.21–4.47)[g]	2.16 (1.08–4.30)[g]
	II	234	19	73	0.77 (0.38–1.58)	0.70 (0.32–1.50)
	III	348	24	76	1.00	1.00
holoTC (n = 839), pmol/L	I	41	35	54	4.15 (1.98–8.69)[g]	4.36 (1.97–9.69)[g]
	II	72	32	72	2.55 (1.24–5.26)[g]	3.01 (1.37–6.64)[g]
	III	120	15	82	1.00	1.00
MMA (n = 839), μmol/L	III	0.56	46	50	3.54 (1.76–7.14)[g]	3.41 (1.63–7.10)[g]
	II	0.28	19	81	1.06 (0.50–2.26)	1.18 (0.54–2.58)
	I	0.20	17	77	1.00	1.00
tHcy (n = 839), μmol/L	III	21.2	39	48	4.94 (2.38–10.29)[g]	4.85 (2.24–10.53)[g]
	II	13.9	29	67	3.03 (1.43–6.33)[g]	2.85 (1.28–6.33)[g]
	I	10.3	14	93	1.00	1.00
Wellness (n = 839)	I	−0.75	38	50	5.10 (2.61–10.46)[g]	5.60 (2.61–12.01)[g]
	II	0.056	26	71	1.72 (0.78–3.80)	1.72 (0.78–3.80)
	III	0.56	14	87	1.00	1.00
TotalTC (n = 839), pmol/L	I	790	26	82	1.00	1.00
	II	1000	27	69	1.22 (0.63–2.37)	1.12 (0.55–2.27)
	III	1300	29	57	1.41 (0.72–2.75)	1.34 (0.67–2.70)
TCsat (n = 839), pmol/L	I	4.0	36	55	3.12 (1.56–6.23)[g]	3.01 (1.44–6.29)[g]
	II	7.2	28	66	2.29 (1.13–4.61)[g]	2.66 (1.26–5.62)[g]
	III	12	18	87	1.00	1.00
TotalHC (n = 839), pmol/L	I	350	37	71	1.07 (0.54–2.15)	1.12 (0.54–2.29)
	II	460	32	73	1.26 (0.65–2.47)	1.07 (0.53–2.17)
	III	620	24	64	1.00	1.00
corHC (n = 163), pmol/L	I	310	29	25	3.19 (1.71–5.96)[g]	3.31 (1.75–6.26)[g]
	II	400	25	29	1.51 (0.77–3.00)	1.35 (0.67–2.71)
	III	540	26	29	1.00	1.00
B12-HC (n = 163), pmol/L	I	140	33	21	2.52 (1.16–5.47)[g]	2.11 (0.92–4.86)[g]
	II	200	26	29	1.35 (0.63–2.90)	1.31 (0.57–3.01)
	III	290	21	33	1.00	1.00
aHC (n = 163), pmol/L	III	280	27	27	0.73 (0.33–1.62)	0.61 (0.26–1.43)
	II	200	29	25	1.10 (0.52–2.33)	0.87 (0.39–1.98)
	I	150	24	31	1.00	1.00
B12-HC/aHC (n = 163), fraction	I	0.68	35	20	3.23 (1.47–7.10)[g]	3.29 (1.41–7.67)[g]
	II	0.96	26	28	1.56 (0.72–3.41)	1.63 (0.69–3.83)
	III	1.6	19	35	1.00	1.00

[a] OR and 95% CI values were previously reported for a different subset of these data Hin et al. *(1)*.
[b] The mean values were calculated for all participants in each tertile independent of the MMSE scores.
[c] There are 82 cases (MMSE <22) in the entire data set (n = 839), but owing to lack of serum material to do the complete analysis, 80 cases are included in the case-control study (n = 163).
[d] There are 208 controls (MMSE >28) in the entire data set (n = 839), and 83 sex- and age-matched controls the case-control study.
[e] OR are adjusted for sex and age as a nonlinear trend and model cases (MMSE <22) vs controls (MMSE >28). The tertile reflecting the best cognitive status is used as 1.0 for all parameters.
[f] ORs adjusted for age, sex, diabetes, cardiovascular diseases, depression, smoking, and treatment with H2-antagonists as a nonlinear trend and models cases (MMSE <22) vs controls (MMSE >28). The tertile reflecting the best cognitive status is designated as the reference group.
[g] CI >1.0; significant associations.

ated with excellent, normal, transient, or deficient B_{12} status. However, all the peaks of the current study were slightly shifted to higher values of both the B_{12}-related variable *x* (mean +12) and the metabolite-related variable *y* (average +0.08). The stronger associations with adverse metabolite status probably reflected the older age of this study population than in the previous study (mean age: 75 vs 63 years *(14)*.

According to the plotted cumulative wellness score (Fig. 1D), participants with cognitive impairment had a higher frequency of low B_{12} status compared with those having MMSE >28 (34% vs 10%). By using a wellness score in future prospective studies, it may be possible to distinguish between those with cognitive impairment influenced by B_{12} status and those whose cognitive impairment is independent of B_{12} status.

The combination of B_{12}, holoTC, MMA, and tHcy in a wellness score showed that individuals whose wellness scores were in the lower third had a 5.1 times higher risk of cognitive impairment (Table 3), which is stronger than the risk observed for all individual parameters. The association of cognitive impairment with high tHcy (OR 4.9) was similar, but tHcy is influenced by other factors than B_{12} status, including poor folate status and increased creatinine. Our data suggest that the wellness score is a stronger predictor of cognitive impairment than the individual markers of B_{12} status, although the 95% CIs for these ORs overlap. Moreover, the association of cognitive impairment with a wellness score was independent of diabetes, cardiovascular diseases, depression, smoking, and treatment with H2 antagonists (Table 3). Application of the wellness score in other studies could be used to identify individuals with a low score that may be related to low B_{12} status in particular.

HoloTC AND B_{12}-HC CORRELATE TO COGNITIVE IMPAIRMENT AND TO EACH OTHER

It has previously been shown that holoTC and B_{12} display strong positive correlations with MMSE *(1)* (Table 2). Here, we found that TCsat and B_{12}-HC also correlate with MMSE. TCsat is expected to correlate with MMSE similarly to holoTC, because holoTC—the amount of B_{12} available for cellular uptake *(11)*—is included in this value. In contrast, B_{12}-HC is not directly available to all cells *(18, 20)*. The strong correlation of B_{12}-HC with MMSE is therefore likely to be indirect. The most obvious explanation might be that B_{12}-HC reflects or is determined by the total load of B_{12} in the individual. B_{12} is released from the cells into the bloodstream as a free molecule *(21)*. Here, HC and TC compete for binding to B_{12}, and if total B_{12} is reduced, both holoTC and B_{12}-HC are reduced. Consistent with this explanation we found a strong correlation between holoTC and B_{12}-HC (Table 2).

The correlation of cognitive impairment with the binding of B_{12} to 1 of the 2 B_{12}-binding proteins was associated with the B_{12} molecule but not with the binder itself, because neither totalTC nor totalHC was correlated with cognitive impairment.

ANALOGS ON HC ARE NOT RELATED TO COGNITIVE IMPAIRMENT

The results of the present study demonstrated no significant differences in the concentrations of analogs between cases with low cognitive function (MMSE <22) compared with age- and sex-matched controls (MMSE >28). Our observation was consistent with results of a previous case-control study of Alzheimer disease *(22)*. The present study demonstrated no difference in circulating concentrations of analogs between cases and controls. Owing to the general decrease in B_{12} and therefore also in B_{12}-HC in cases, we observed a significant decrease in the B_{12}-HC/aHC ratio in cases compared with controls.

Moreover, the present study demonstrated a strong correlation between aHC and totalHC, a result consistent with previously reported findings *(19)*. It is possible that these associations may be explained by the molecular dynamics in the blood. The rate of the analogs binding to HC is proportional to totalHC, and if the latter were reduced, the absolute amount of analogs on HC might be expected to decrease.

In summary, in the present study we demonstrated that combined use of B_{12}, holoTC, MMA, and tHcy identified a large subgroup of individuals with cognitive impairment who appear to suffer from a poor B_{12} status. These results suggest that using the wellness score may identify high-risk individuals with low vitamin B_{12} status. The present study showed no association of B_{12}-binding proteins or analogs with cognitive impairment.

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