Plasma Total Homocysteine and Memory in the Elderly: The Hordaland Homocysteine Study

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We examined the relation between plasma total homocysteine (tHcy), folate, vitamin B12, and episodic memory in elderly community-dwelling subjects. A population-based study was conducted in 1992 and 1993, and subjects were re-investigated after 6 years. Plasma analytes were determined on both occasions. At follow-up, memory performance, using the Kendrick Object Learning Test, was investigated in 2,189 subjects (age, 65–67 years at baseline). Subjects with memory deficit (test score, <25) had higher tHcy and lower folate at follow-up compared with those without memory deficit: 12.6 (95% confidence interval [CI], 12.1, 13.1) versus 11.5 (95% CI, 11.3, 11.6) μmol/L ($p < 0.001$) for tHcy, and 6.7 (95% CI, 6.2, 7.1) versus 7.6 (95% CI, 7.5, 7.8) nmol/L ($p < 0.001$) for folate. The risk of memory deficit increased according to quintiles of tHcy both at baseline and at follow-up. A decline in tHcy, or an increase in folate, over a 6-year period was associated with a higher memory test score; and vice versa. These findings indicate that increased plasma tHcy is an independent risk factor for memory deficit both cross-sectionally and prospectively, and that a "favorable" change in folate or tHcy concentrations over time is associated with better memory performance.

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Increased levels of plasma total homocysteine (tHcy) are found in a variety of disorders[1] and are common in elderly people,[2,3] especially in those with psychogeriatric syndromes.[4,5] Increased tHcy levels occur more frequently in patients with Alzheimer's disease (AD)[6,7] or with vascular dementia[6,8] compared with age-matched control subjects; many subsequent studies have confirmed these associations (see Selhub and colleagues,[3] Smith,[9] and Morris[10]).

A prospective study in dementia patients found that increased tHcy levels are associated with more rapid progression of the disease,[6] and a large-scale community study showed that increased tHcy levels up to 11 years before diagnosis are associated with an increased risk for development of dementia.[11] The latter observation raises the question of whether increased tHcy levels trigger the cognitive decline that is often a precursor of dementia, and whether reducing the levels of tHcy in those at risk could prevent the development of dementia. In the absence of results from intervention trials with tHcy-reducing treatments (such as B vitamins) in cognitively impaired subjects, we can approach these questions through studies on the elderly in the community. Several studies in community-dwelling elderly subjects have reported relations between impaired memory and other cognitive domains and increased levels of tHcy.[12–23] Possible physical substrates for the cognitive deficit associated with tHcy are brain atrophy, notably of the medial temporal lobe,[24–26] and damage to the white matter in the brain.[27,28]

Increased plasma tHcy concentration is a sensitive marker of folate and vitamin B_{12} status.[1] B vitamins are required for well-being and normal functioning of the brain,[29] but concentrations of B vitamins are frequently inadequate in late life.[1,22,30] A positive relation has been reported between various cognitive tests and intake or blood concentrations of B vitamins.[12,16,18,29,31,32] Vitamin deficiency may lead to memory problems, cognitive decline, and even dementia,[1,30] and there is evidence that AD and vascular dementia are related to blood concentrations of B vitamins, as well as tHcy.[1,10]

The Hordaland Homocysteine Study[33] provides an

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opportunity to study associations among memory, tHcy, and some of its determinants. In this cohort, blood samples were collected in 1992 and 1993 and again in 1997 to 1999, and memory testing among 2,189 elderly subjects (born 1925–1927) was performed in 1997 through 1999. In this study, we have examined the following questions: (1) Is there any relation between memory test scores and tHcy, folate, and vitamin B_{12} levels measured at the time of memory assessment?; (2) Is there any relation between memory test scores and tHcy, folate, and vitamin B_{12} levels measured 6 years before memory assessment?; (3) Is there any relation between memory test scores and changes in the levels of tHcy, folate, and vitamin B_{12} that occurred during the previous 6 years? The latter question is relevant to the possible causative role of tHcy: The hypothesis under scrutiny is that increasing levels of tHcy over time might be associated with poorer memory, whereas decreasing levels of tHcy might be associated with better memory.

Subjects and Methods

Study Population

The Hordaland Homocysteine Study[33] was conducted as a collaboration among the University of Bergen, local health services, and the National Health Screening Service (now the Norwegian Institute of Public Health). Recruitment of the study sample (the Cognitive Sub-study of the Hordaland Homocysteine Study) is described on the Web (available at: www.uib.no/isf/husk/Vedlegg_dokumenter/Cognitive_Sub_study.pdf). The Cognitive Sub-study was confined to those living in the city of Bergen who were born between 1925 and 1927. A total of 2,841 elderly subjects attended both the baseline (1992–1993) and the follow-up (1997–1999) studies and were invited to participate in cognitive tests; 2,197 (77.3%) of these subjects agreed. Those subjects who had a tHcy concentration of 40μmol/L or more at baseline (n = 8) were excluded because they had been treated with cobalamin or folic acid after the first survey, leaving 2,189 subjects for analysis. All participating subjects gave their written, informed consent. The study protocol was approved by the Regional Committee for Medical Research Ethics of Western Norway.

Data Collection

At baseline, participants underwent the standard cardiovascular examinations of the National Health Screening Service.[34] Several self-administered questionnaires focusing on cardiovascular risk factors, lifestyle factors, and dietary habits were used. Details on the baseline data collection are reported elsewhere.[35] The follow-up examination included essentially the same variables. In this study, a history of cardiovascular disease (CVD) was defined as self-reported CVD (myocardial infarction, angina pectoris, stroke, thrombosis, phlebitis) at baseline or follow-up or a hospitalization record of CVD during follow-up from 1992 to 1998. The criteria for a hospitalization record were based on the main discharge diagnosis according to the *International Classification of Diseases, Ninth Revision* (ICD-9) and have been described in detail previously.[36] Subjects who had not reported their CVD history in the questionnaires and those without a hospitalization record (n = 148) were excluded from statistical analyses when CVD history was in the model. A history of hypertension was defined as current or previous use of antihypertensive drugs and was based on self-reported data collected at follow-up. Those who had not reported their hypertension history (n = 46) were excluded from statistical analyses when hypertension history was in the model.

Those who reported daily smoking of cigarettes, cigars, cigarillos, or a pipe were considered as smokers in both surveys. For this study, consumption of caffeine-containing coffee was divided into two groups: up to four cups, and five or more cups per day.

The follow-up study included assessment of anxiety and depression using the Hospital Anxiety and Depression Scale (HADS),[37,38] which consists of two 7-item subscales, HADS-A for anxiety and HADS-D for depression. HADS-A contains items mainly related to restlessness and worry, and one item reflects panic attacks. HADS-D focuses mainly on the reduced pleasure response aspect (anhedonia) of depression, but it also includes psychomotor retardation and impaired mood.

Memory Assessment

The follow-up examination included the Kendrick Object Learning Test (KOLT).[39] The KOLT is commonly used in Norway[40] and is designed to assess dementia status and episodic memory performance among noninstitutionalized elderly people. The KOLT has been validated for the detection of memory impairment in old age[40] and for distinguishing patients with mild AD from other forms of mental impairment.[41] Four cards with 10, 15, 20, and 25 pictures are shown individually for 30, 45, 60, and 75 seconds. When each card is taken away, the subject is asked to name as many pictures on the card as he or she can remember. A possible maximum KOLT score is 70. A score of 20 or less is categorized as severely memory impaired or demented, whereas a score of 21 to 25 is considered moderately memory impaired. Using the KOLT, the KOLT is valuable for community studies because it is normally distributed, and thus does not show a ceiling effect. The number of severely memory-impaired participants in this study was rather small. We therefore combined the groups of moderately (n = 161; 7.4%) and severely memory-impaired (n = 74; 3.4%) participants and defined this group (n = 235; 10.7%) as subjects with memory deficit (score, ≤25).

Plasma Measurements

Nonfasting EDTA blood samples were collected for determination of tHcy, B vitamins, and gene polymorphisms. The EDTA sample was kept cool until centrifugation took place. The baseline plasma and blood samples were stored at −20°C, whereas the follow-up samples were stored at −80°C.

Plasma tHcy concentration was determined using a fully automated high-performance liquid chromatography assay.[42,43] The duration of storage ranged from a few days to

up to 6 months for baseline samples and up to 18 months for follow-up samples.

Folate and vitamin B_{12} levels were determined by microbiological assays on microtiter plates.[44,45] Before baseline folate and vitamin B_{12} measurements were performed, the plasma samples had been stored at −20°C for up to 10 years. Follow-up vitamin measurements were performed in plasma stored at −80°C for up to 12 months. Plasma folate declines during storage, but the change is independent of the folate concentration.[46] Because we had measured folate in a subset from the baseline visit in 1995, and then reanalyzed the same samples in 2000, we were able to allow for this decline by using a correction factor (see Nurk and colleagues[47] for further details).

Samples used for the preparation of serum were collected into an evacuated tube containing sodium sulphite titration gel and were centrifuged within 2 hours. The serum tubes were transported to the Department of Clinical Chemistry, Ullevål Hospital, Oslo, Norway, for measurements of lipid-related factors and creatinine. Creatinine was measured in the follow-up samples only, using the alkaline picrate method (Roche, Basel, Switzerland) run on a Hitachi 917 auto analyzer (Roche Diagnostic, Switzerland).

The methylenetetrahydrofolate reductase (MTHFR) 677C→T and 1298A→C polymorphisms were determined in the packed cell fraction of blood from the baseline samples using real-time polymerase chain reaction or mutagenically separated polymerase chain reaction and multiple-injection capillary electrophoresis.[48,49] Apolipoprotein E (ApoE) genotypes were determined using a one-stage polymerase chain reaction method using restriction enzyme *Cfo*I and polyacrylamide gel electrophoresis.[50]

Statistical Analyses

The distributions of plasma concentrations of tHcy, folate, and vitamin B_{12} were markedly skewed, and log-transformed data were used in all analyses, except for changes in plasma tHcy, folate, and vitamin B_{12} concentrations over the 6-year period, which are presented as arithmetic values. For comparison between groups, the independent sample t test, the χ^2 test, or analysis of variance was used. Multiple logistic regression analyses (adjusted for sex, ApoE ε4 alleles, education, history of CVD/hypertension, and, at follow-up, depression score as well) were used to estimate the associations between quintiles of tHcy, folate, and vitamin B_{12} concentrations in plasma and memory deficit. We repeated these analyses by adding risk factors that are known to influence tHcy status (smoking, coffee consumption, MTHFR 677C→T polymorphism, and, at follow-up, creatinine concentration and intake of B-vitamin–containing supplements) in the model. Because the mean plasma levels of tHcy, folate, and vitamin B_{12} differed significantly between men and women, sex-specific quintiles were used.

The average of baseline and follow-up values of plasma tHcy, folate, and vitamin B_{12} were calculated for each subject to study dose–response relations for exposure to the analytes. Gaussian generalized additive regression models, as implemented in S-PLUS 6.2 for Windows (Insightful Corporation, Seattle, WA), were used to generate graphic representations of the dose–response relations, adjusted for sex. On the y-axis, the model generates a reference value of zero that approximately corresponds to the KOLT value associated with the mean of the average tHcy or B-vitamin concentrations for all subjects. A similar approach was used to generate dose–response curves for the change in tHcy or folate over 6 years versus the KOLT score (adjusted for sex and the relevant baseline analyte concentration). Linear regression analyses were used to examine significant associations between the KOLT score and averages of the baseline and follow-up concentrations of analytes and between the KOLT score and changes of analytes over 6 years. Except generalized additive models, all statistical analyses were performed using the Statistical Package for the Social Sciences 12.0 for Windows (SPSS, Chicago, IL). p values less than 0.05 were considered significant.

Results

Demographics, Blood Indices, and Genetics

Characteristics of the study population at follow-up by memory status, as well as changes in tHcy, folate, and vitamin B_{12} from baseline to follow-up, are presented in Table 1. The proportion of men and subjects with low education (≤9 years), depression, or history of CVD were significantly greater among subjects with memory deficit than among those without deficit. Memory-impaired subjects had significantly greater plasma tHcy and creatinine concentrations and significantly lower plasma folate concentration than those without memory deficit and tended to have a lower use of B-vitamin–containing supplements. The prevalence of ApoE ε4 allele was also greater among those with memory deficit.

Memory Function in Relation to Total Homocysteine and B-Vitamin Concentrations at Baseline

Mean tHcy concentration at baseline was greater among those with memory deficit (12.0μmol/L; 95% confidence interval [CI], 11.6, 12.4) compared with those without (11.2μmol/L; 95% CI, 11.1, 11.3; $p < 0.001$). Folate concentration was significantly lower among subjects with memory deficit (5.9nmol/L; 95% CI, 5.6, 6.3) than among subjects without deficit (6.7nmol/L; 95% CI, 6.5, 6.8) ($p < 0.001$). There were no statistically significant differences in vitamin B_{12} concentrations between these two groups (328pmol/L [95% CI, 312, 345] vs 343pmol/L [95% CI, 337, 349]) ($p = 0.10$).

Sex Difference

We found a significant sex difference in KOLT scores (Table 2). Men had a lower mean KOLT score than women. Similarly, the proportions of moderate (KOLT score, 21–25) and severe (KOLT score, ≤20) memory deficit were significantly greater among men compared with women. Several other factors differed between the sexes, including educational level, coffee consumption, history of CVD/hypertension, anxiety, creatinine, B vitamins, and tHcy itself (data not

Table 1. Characteristics of the Study Population at Follow-up (1997-1999) and Changes in Selected Variables from Baseline (1992-1993) to Follow-up: The Hordaland Homocysteine Study

Characteristic	Total Subjects (N),[a] KOLT >25/≤25	No. of Subjects (%) or Mean (95% CI)		p[b]
		KOLT >25	KOLT ≤25	
Age, yr	1,954/235	72.0 (71.9–72.0)	72.0 (71.9–72.1)	0.73
Male sex	1,954/235	842 (43.1)	141 (60.0)	<0.001
Coffee consumption ≥5 cups a day	1,950/234	294 (15.1)	40 (17.1)	0.48
Daily smokers	1,936/233	277 (14.3)	32 (13.7)	0.88
Education ≤ 9yr	1,920/231	757 (39.4)	117 (50.6)	0.001
HADS-D score	1,690/189	3.4 (3.3–3.5)	4.4 (3.9–4.8)	<0.001
HADS-A score	1,596/171	4.0 (3.9–4.2)	4.3 (3.8–4.8)	0.22
History of CVD	1,824/217	621 (34.0)	103 (47.5)	<0.001
History of hypertension	1,911/232	644 (33.7)	86 (37.1)	0.34
Vitamin supplements,[c] days/yr	1,813/202	76 (69–82)	57 (40–74)	0.06
Plasma tHcy,[d] μmol/L	1,950/234	11.5 (11.3–11.6)	12.6 (12.1–13.1)	<0.001
Change in tHcy,[e] μmol/L	1,950/234	0.4 (0.2–0.5)	0.7 (0.2–1.2)	0.14
Plasma folate,[d] nmol/L	1,940/233	7.6 (7.5–7.8)	6.7 (6.2–7.1)	<0.001
Change in folate,[e] nmol/L	1,940/233	1.7 (1.3–2.0)	1.1 (0.4–1.8)	0.25
Plasma vitamin B_{12},[d] pmol/L	1,947/234	347 (341–354)	343 (325–363)	0.71
Change in vitamin B_{12},[e] pmol/L	1,945/234	8 (−8 to 24)	13 (−47 to 72)	0.86
Serum creatinine, mmol/L	1,953/235	93 (92 to 93)	95 (93 to 97)	0.039
ApoE ε4, one allele	1,939/235	555 (28.6)	73 (31.1)	0.47
ApoE ε4, two alleles	1,939/235	47 (2.4)	22 (9.4)	<0.001
MTHFR 677 CT genotype	1,954/235	813 (41.6)	98 (41.7)	0.97
MTHFR 677 TT genotype	1,954/235	159 (8.1)	19 (8.1)	0.90
MTHFR 1298 AC genotype	1,948/234	859 (44.1)	105 (44.9)	0.87
MTHFR 1298 CC genotype	1,948/234	231 (11.9)	31 (13.2)	0.64
Compound heterozygosity MTHFR 677CT + 1298AC	1,948/234	380 (19.5)	50 (21.4)	0.55

[a]The sample number varies slightly among the reported variables according to different numbers of missing data.
[b]Independent sample t or χ^2 test.
[c]Intake of multivitamin supplements or any type of B vitamin supplements.
[d]Geometric mean.
[e]Change over 6-year period.

CI = confidence interval; KOLT = Kendrick Object Learning Test; HADS-A = Hospital Anxiety and Depression Scale—anxiety subscale; HADS-D = Hospital Anxiety and Depression Scale—depression subscale; CVD = cardiovascular disease; tHcy = total homocysteine; ApoE = apolipoprotein E; MTHFR = methylenetetrahydrofolate reductase.

shown). Nevertheless, the sex difference in the KOLT score remained significant after adjusting for these factors (see Table 2). Because the observed patterns of associations of the KOLT score with tHcy, folate, and B_{12} (see descriptions later in this article) levels were similar in men and women, we present combined data but have adjusted for sex.

Total Homocysteine and B-Vitamin Status as Risk Factors for Memory Deficit

We investigated the risk for memory deficit according to quintiles of tHcy or B-vitamin concentrations (Table 3). The highest quintiles of tHcy, both at baseline and at follow-up, were associated with increased risk for memory deficit at follow-up. The association was

Table 2. Effects of Sex on Kendrick Object Learning Test Score: The Hordaland Homocysteine Study

KOLT Results	Men (n = 983)	Women(n = 1206)	p[a]
Mean score (95% CI)	33.3 (32.8–33.8)	36.7 (36.2–37.2)	<0.001
Mean adjusted[b] score (95% CI)	33.4 (32.8–34.0)	37.5 (36.9–38.0)	<0.001
Score ≤ 25, n (%)	141 (14.3)	94 (7.8)	<0.001
Score = 21–25, n (%)	94 (9.6)	67 (5.6)	<0.001
Score ≤ 20, n (%)	47 (4.8)	27 (2.2)	0.001

[a]Independent sample t or χ^2 test.
[b]Adjusted for factors for which significant sex differences were observed: coffee consumption of five or more cups per day; education of 9 years or less; anxiety; history of cardiovascular disease and hypertension; plasma total homocysteine, folate, and vitamin B_{12}; and serum creatinine. n = 787 men; n = 886 women.

KOLT = Kendrick Object Learning Test; CI = confidence interval.

Table 3. Risk Ratios for Memory Deficit (Kendrick Object Learning Test score ≤ 25) by Quintiles of Plasma Total Homocysteine, Folate, and Vitamin B_{12}: The Hordaland Homocysteine Study

Analyte at different timepoints	Quintile	Median	Memory Deficit, n (%)	Adjusted for Sex		Multiple Adjusted[a]	
				OR (95% CI)	*p* for trend	OR (95% CI)	*p* for trend
tHcy							
Baseline	1	8.3μmol/L	38 (8.6)	1.00		1.00	
	2	9.9μmol/L	45 (9.6)	1.11 (0.71–1.75)		1.17 (0.71–1.92)	
	3	11.1μmol/L	31 (7.9)	0.92 (0.56–1.52)		0.98 (0.57–1.69)	
	4	12.5μmol/L	54 (12.5)	1.47 (0.95–2.29)		1.40 (0.85–2.29)	
	5	15.4μmol/L	67 (14.8)	1.89 (1.23–2.88)	0.001	1.87 (1.18–2.98)	0.004
Follow-up	1	8.3μmol/L	33 (7.3)	1.00		1.00	
	2	10.0μmol/L	37 (8.4)	1.21 (0.74–1.97)		1.05 (0.58–1.89)	
	3	11.5μmol/L	50 (10.6)	1.56 (0.98–2.48)		1.70 (1.01–2.88)	
	4	13.3μmol/L	51 (13.2)	1.97 (1.24–3.13)		1.66 (0.95–2.91)	
	5	16.5μmol/L	63 (14.6)	2.24 (1.43–3.50)	<0.001	2.34 (1.39–3.91)	<0.001
Folate							
Baseline	1	3.7nmol/L	63 (14.3)	1.79 (1.16–2.76)		1.64 (1.02–2.63)	
	2	5.1nmol/L	52 (12.4)	1.47 (0.94–2.31)		1.51 (0.93–2.44)	
	3	6.5nmol/L	39 (9.2)	1.11 (0.69–1.78)		1.07 (0.64–1.78)	
	4	8.2nmol/L	43 (9.2)	1.08 (0.68–1.72)		0.96 (0.57–1.60)	
	5	11.7nmol/L	37 (8.6)	1.00	0.002	1.00	0.006
Follow-up	1	4.0nmol/L	50 (14.7)	2.26 (1.43–3.58)		1.93 (1.11–3.36)	
	2	5.4nmol/L	42 (10.2)	1.49 (0.93–2.39)		1.36 (0.78–2.39)	
	3	6.7nmol/L	61 (12.3)	1.78 (1.15–2.76)		1.95 (1.18–3.25)	
	4	8.5nmol/L	45 (10.0)	1.43 (0.90–2.27)		1.56 (0.91–2.67)	
	5	14.5nmol/L	35 (7.3)	1.00	0.001	1.00	0.056
Vitamin B_{12}							
Baseline	1	222pmol/L	52 (12.6)	1.59 (1.01–2.49)		1.63 (1.00–2.64)	
	2	287pmol/L	54 (11.6)	1.42 (0.91–2.23)		1.26 (0.77–2.05)	
	3	340pmol/L	49 (11.0)	1.31 (0.83–2.07)		1.30 (0.80–2.12)	
	4	404pmol/L	44 (9.9)	1.17 (0.73–1.86)		1.08 (0.65–1.79)	
	5	522pmol/L	36 (8.6)	1.00	0.028	1.00	0.042
Follow-up	1	220pmol/L	47 (10.7)	0.94 (0.61–1.44)		0.90 (0.55–1.48)	
	2	289pmol/L	63 (13.7)	1.26 (0.84–1.88)		1.27 (0.80–2.01)	
	3	341pmol/L	39 (9.2)	0.78 (0.50–1.22)		0.74 (0.44–1.24)	
	4	410pmol/L	36 (8.4)	0.74 (0.47–1.17)		0.67 (0.39–1.15)	
	5	537pmol/L	49 (11.5)	1.00	0.39	1.00	0.48

[a]Adjusted for sex, apolipoprotein E ε4 alleles, education, and history of cardiovascular disease and hypertension. In addition, the results at follow-up were adjusted for depression score.

OR = odds ratio; CI = confidence interval; tHcy = total homocysteine.

strongest with follow-up tHcy measurements and remained significant after additional adjustment for ApoE ε4 genotype and for multiple other factors known to influence cognition. When plasma folate and vitamin B_{12} concentrations were added to the model, the associations weakened marginally and remained significant for the follow-up data (p for trend = 0.002), but were no longer quite significant for the baseline data (p for trend = 0.056) (data not shown). Furthermore, when risk factors known to influence tHcy status (smoking, coffee consumption, MTHFR 677C→T polymorphism, and, at follow-up, creatinine concentration and intake of B-vitamin–containing supplements) were additionally included in the model, the results changed only marginally: p for trend = 0.001 at follow-up, and p for trend = 0.03 at baseline.

The lowest quintiles of folate concentration and trend over quintiles were significantly associated with increased risk for memory deficit at both time points, when adjusted for sex alone and after adjustment for multiple factors (see Table 3). At follow-up, the risk for memory deficit was 93% greater for subjects in the lowest quintile of folate, and the trend was borderline significant when controlling for multiple risk factors. Additional adjustment for plasma tHcy and vitamin B_{12} concentrations further weakened the trend at baseline (p = 0.052), whereas the trend at follow-up was not significant (p for trend = 0.44) (data not shown).

We found a trend for increasing risk for memory deficit with decreasing quintiles of baseline vitamin B_{12} concentrations at baseline, and that those in the lowest quintile of vitamin B_{12} had an increased risk (see Table 3). In contrast with tHcy and folate, vitamin B_{12} level

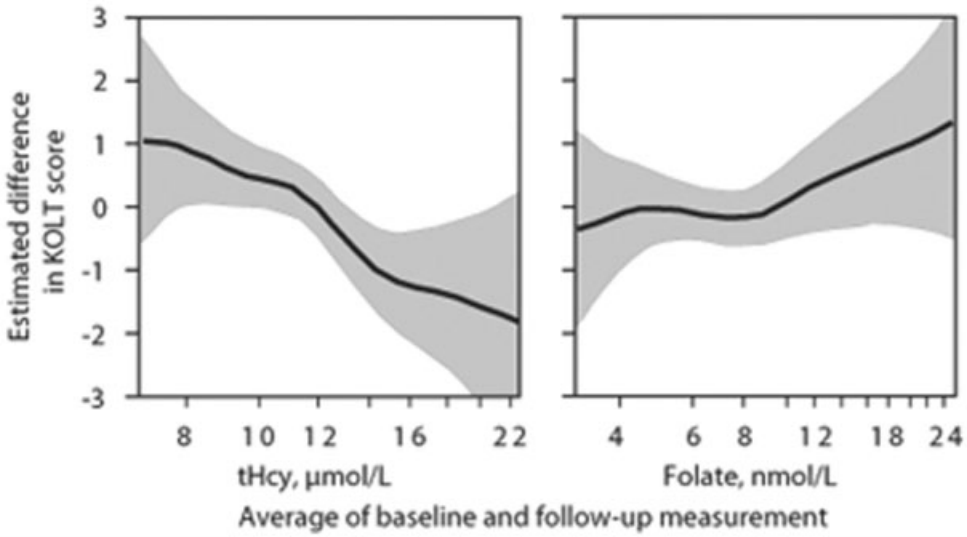

Fig 1. Associations between Kendrick Objective Learning Test (KOLT) score at follow-up and the average, in individual subjects, of the baseline and follow-up concentrations of plasma total homocysteine (tHcy) (n = 1,670) and folate (n = 1,660), adjusted for sex, apolipoprotein E (ApoE) ε4, education, cardiovascular disease (CVD), and depression. The reference value for KOLT is the approximate value associated with the mean of the average tHcy and folate concentrations for all subjects. Solid lines *are the estimated dose–response curves;* dashed lines *are the 95% confidence intervals. The lowest and highest 1 percentile of tHcy and folate concentrations are not included.*

was not significantly associated with memory deficit at follow-up.

Dose–Response Relations

As a measure of exposure to tHcy and B vitamins over the 6-year period, the average of baseline and follow-up values of plasma tHcy, folate, and vitamin B_{12} were calculated for each subject. Linear regression analysis, adjusting only for sex, indicated that the averages of the baseline and follow-up concentrations of analytes were significantly related to the KOLT score for both tHcy (N = 2,189; $p < 0.001$) and folate (N = 2,173; $p = 0.006$), but not for vitamin B_{12} ($p = 0.57$). Plasma tHcy was inversely associated and folate was positively associated with the KOLT score. Graphic representations of how the KOLT score varied as a function of average tHcy or folate concentrations are presented in Figure 1, where the associations are adjusted for sex, ApoE ε4, education, CVD, and depression. Linear regression analysis for the fully adjusted relations were significant for tHcy (n = 1,670; $p = 0.001$), but not for folate (n = 1,660; $p = 0.12$). The association between average tHcy concentration and KOLT score remained significant when factors affecting tHcy concentration, including intake of B vitamin–containing supplements, were also controlled for (n = 1,650; $p = 0.002$).

As a way of assessing the possible influence on memory of changes over time in the levels of tHcy and the vitamins, the differences in the KOLT score as a function of changes in plasma tHcy or vitamin concentrations over the 6-year period from baseline to follow-up were determined. A decline in tHcy levels over 6 years was associated with a greater KOLT score at follow-up, whereas an increase in tHcy levels was associated with a lower KOLT score. Consistent with this result, an increase in folate levels was associated with a higher memory score, whereas a decline in folate levels resulted in a lower score. Linear regression analyses, adjusting for sex and baseline analyte concentration, were significant for changes in tHcy (N = 2,189; $p = 0.003$) and folate (N = 2,173; $p = 0.026$), but not vitamin B_{12} ($p = 0.17$). The relations are shown graphically in Figure 2, adjusted for sex, baseline analyte level, ApoE ε4, education, CVD, and depression. Linear regression analysis, including all those covariates, showed that the association of KOLT score with changes in tHcy remained significant (n = 1,670; $p = 0.003$), whereas that for folate became marginal (n = 1,660; $p = 0.056$). Inclusion of risk factors affecting tHcy status, including intake of B-vitamin–containing supplements, in the model did not take away the relation between the KOLT score and change in tHcy concentration (n = 1,650; $p = 0.011$).

We also examined the relation between change in tHcy concentration over the 6-year period and the KOLT score at follow-up in those subjects who were not memory impaired (KOLT score, >25). In this latter group, there was also an inverse relation between change in tHcy concentration and KOLT score (n = 1,497; $p = 0.016$, adjusted for sex, education, ApoE4, depression, and CVD/hypertension) that became bor-

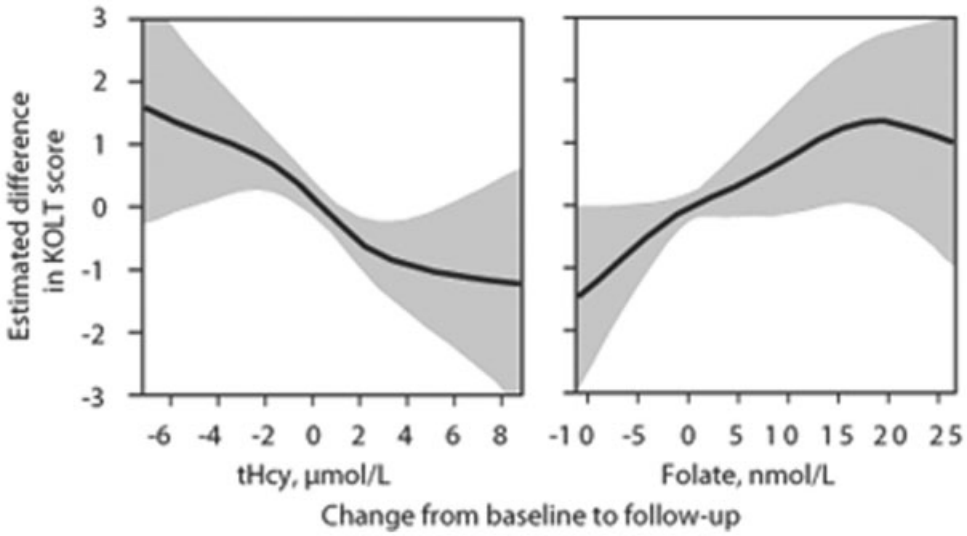

Fig 2. Associations between the Kendrick Objective Learning Test (KOLT) score at follow-up and changes in plasma concentrations of total homocysteine (tHcy) (n = 1,670) and folate (n = 1,660) between baseline and follow-up. The relations were adjusted for sex, apolipoprotein E (ApoE) ε4, education, cardiovascular disease (CVD), depression, and baseline tHcy for the tHcy plot, and for sex, ApoE ε4, education, CVD, depression, and baseline folate for the folate plot. The reference value for KOLT is the approximate value associated with no change over the 6-year period in tHcy or folate levels, respectively. Solid lines *are the estimated dose–response curves;* dashed lines *are the 95% confidence intervals. The lowest and highest 1 percentile of tHcy and folate concentrations are not included.*

derline significant after additional adjustment for smoking, coffee consumption, MTHFR 677C→T polymorphism, creatinine concentration, and intake of B vitamin–containing supplements (n = 1,481; p = 0.08).

Methylenetetrahydrofolate Reductase Polymorphisms

Plasma tHcy and folate levels were significantly different in subjects with different MTHFR 677C→T genotypes, as reported previously for the Hordaland Homocysteine Study.[51] Neither the MTHFR 677C→T nor the MTHFR 1298A→C polymorphism was significantly associated with the KOLT score, and further stratification by increased tHcy or low vitamin levels did not result in significant associations of MTHFR polymorphisms with KOLT score (data not shown).

Comparisons between Subjects Who Did and Subjects Who Did Not Undergo Cognitive Testing

We searched for potential differences between these two groups in two ways. First, we compared attenders with nonattenders of the follow-up study. Second, in those who attended, we compared the elderly subjects who underwent the KOLT with those who did not (Table 4). Individuals who did not attend had significantly greater mean tHcy and lower vitamin B_{12} concentrations at baseline compared with those who did attend. In addition, several other factors suspected to influence the KOLT score (sex, educational level, CVD, and ApoE ε4 allele frequency) differed significantly between subjects who did not attend at follow-up and those who did attend. In contrast, there were no significant differences in plasma tHcy and vitamins in those who attended the follow-up but did not participate in memory testing compared with those who did undergo testing. However, there were differences for sex, ApoE ε4 allele frequency, and educational level.

Discussion

In a population-based cohort study of 2,189 elderly men and women, we have shown that plasma tHcy concentration was strongly and independently associated with deficit in episodic memory, as assessed by the KOLT. In addition, we found that increased tHcy concentration and low folate and vitamin B_{12} concentrations measured about 6 years before memory testing were associated with increased risk for subsequent memory deficit. Furthermore, "unfavorable" changes in the concentrations of plasma tHcy (ie, an increase) or of folate (ie, a decrease) over the 6-year period were associated with lower KOLT scores.

A strength of our study is the size of the study population and that the design included collection of blood samples at two time points 6 years apart. Memory testing was, however, performed only at the follow-up; therefore, we could not exclude that there was no memory deficit at baseline, nor could we examine change in memory function. However, our finding that the inverse relation between KOLT score and a change

Table 4. Factors That May Influence Kendrick Object Learning Test Score in Attenders and Nonattenders at Follow-up and in Those Attenders Who Were and Those Who Were Not Tested: Cognitive Sub-study of the Hordaland Homocysteine Study

Influential Factors	Number Attending (yes/no)[a]	Attended (mean or %)	Did Not Attend (mean or %)	p[b]	No. Tested with KOLT[a] (yes/no)	Tested (mean or %)[c]	Not Tested (mean or %)[c]	p[b]
Plasma tHcy, μmol/L	2,841/889	11.3	12.2	<0.001	2,192/639	11.6	11.8	0.27
Plasma folate, nmol/L	2,840/889	5.1	5.0	0.18	2,181/636	7.5	7.8	0.18
Plasma vitamin B_{12}, pmol/L	2,839/888	339	327	0.017	2,189/639	348	337	0.13
Male sex, %	2,841/889	43.8	38.5	0.006	2,197/644	45.0	39.6	0.017
Education ≤ 9yr, %	2,781/693	43.2	57.9	<0.001	2,159/622	40.8	50.2	<0.001
ApoE ε4 one or two alleles, %	2,821/885	31.0	36.9	<0.001	2,182/639	32.1	27.1	0.018
CVD/hypertension, %	2,473/714	36.6	48.0	<0.001	1,920/532	57.2	57.5	0.94
HADS-D score					1,886/504	3.5	3.7	0.07

Full details of the recruitment into the Cognitive Sub-study are available on the Web at: www.uib.no/isf/husk/Vedlegg_dokumenter/Cognitive_Sub_study.pdf

[a]The sample number varies slightly among the reported variables according to different numbers of missing data.
[b]Independent sample t or χ^2 test.
[c]Based on follow-up data.

KOLT = Kendrick Object Learning Test; tHcy = total homocysteine; ApoE = apolipoprotein E; CVD = cardiovascular disease; HADS-D = Hospital Anxiety and Depression Scale—depression subscale.

in tHcy level over 6 years was still found in subjects who were not memory impaired at follow-up argues against memory impairment being a cause of the change in tHcy over time. Although the response rate for the follow-up study was relatively high, and a relatively high proportion of the attenders volunteered for cognitive testing, the study population comprised only 59% of those invited at follow-up (more information about the study population is available via the Internet at: www.uib.no/isf/husk/Vedlegg_dokumenter/Cognitive_Sub_study.pdf). Furthermore, comparisons between those who were cognitively tested and those who were not demonstrated several differences (see Table 4). Consequently, it is unlikely that our study population is representative of the general elderly population. In our statistical analyses, we have adjusted for several potential sources of bias, but we cannot exclude residual confounding.

We found that women had significantly higher KOLT scores than men. With the conventional thresholds for KOLT used in Norway, the prevalence of moderate and severe memory impairment was twice as high among men as among women. A few other studies have also reported sex differences in some cognitive tests,[52–55] and notably, women often perform better in tests of episodic memory,[54] as we have found in this population.

The association between increased tHcy and cognitive impairment, dementia and AD is well established,[6–10,29] and several reports have noted an association with memory impairment.[15–19,22] Our study shows that the risk for memory deficit increases significantly with tHcy quintiles, independent of other factors related to cognitive impairment and to tHcy status. Even after including plasma folate and vitamin B_{12} in the model, the associations remained the same. Although the association was strongest for follow-up plasma tHcy concentrations, a significant association was also found with tHcy concentrations measured 6 years before cognitive testing. This result suggests that an increased tHcy level precedes or appears at an early stage of memory decline, which is in agreement with earlier reports on a variety of cognitive tests.[6,11,15,22,23]

That the relation between tHcy and memory function was weaker in the baseline data could be due to the regression dilution phenomenon. In the Hordaland Homocysteine Study, it has been calculated that the 6-year interval between visits will underestimate the true strength of the association with baseline tHcy by about 30%.[56] An additional possible explanation is that tHcy as a risk factor for poor memory performance may become stronger with increasing age.[17] In the Scottish Mental Surveys,[18] survivors from two birth cohorts (1921 and 1936) were studied from 1997 to 1999, and plasma tHcy concentration was inversely related to cognitive function only in the oldest cohort. Other studies investigating relatively young populations, including the Rotterdam Study,[57] among community-dwelling respondents aged 55 years or older, and the Normative Aging Study,[12] which in-

volved participants older than 53 years, also indicate that the associations between tHcy and cognition are weak or absent in these younger age groups. In contrast, in most studies conducted among people with an age range similar our cohort, tHcy is a risk factor for impaired cognition, dementia, or both.[6,11,16,19,21,23,58]

We found that low folate status was associated with memory deficit and lower KOLT score. This confirms findings from other studies in which low folate concentrations are modestly associated with poor performance in memory and other cognitive tests.[6,12,18,22,31,32,59] Compared with plasma tHcy, we found that the associations between folate and memory deficit were less consistent. This result could indicate that plasma tHcy is a better and earlier marker of cognitive impairment than folate. In line with these results, an earlier study[12] has shown that plasma tHcy concentration is more strongly related to spatial copying performance than either folate or vitamin B_{12}. Another possible explanation is related to that plasma folate levels decline during storage.[46] The probable consequence is that the associations are weaker in our study than those found in populations where plasma folate has been measured in fresh samples.

In this study, only the baseline vitamin B_{12} quintiles were associated with memory deficit. This confirms published data, which showed that the associations between vitamin B_{12} and cognitive dysfunction and dementia in older people are weaker than the folate association,[6,22,60,61] and often, the association is absent or nonsignificant.[5,13,21,22,62,63] A better measure of vitamin B_{12} status may be the level of holotranscobalamin, which is an early marker of vitamin B_{12} deficiency.[1] In line with this, the holotranscobalamin level is related to cognitive scores in healthy elderly people,[64,65] and low levels frequently occur in AD.[65]

Homozygosity for the MTHFR 677C→T polymorphism is associated with hyperhomocystinemia, particularly in subjects with low folate status.[1] Thus, theoretically, those with the TT genotype should be at greater risk for development of cognitive impairment. However, most studies have not found significant associations between the MTHFR 677C→T polymorphism and cognitive function.[6,58,66,67] In a few studies, an increased risk for dementia was observed, but only in those who had the TT genotype combined with low folate status or increased tHcy level.[68,69] In this study, neither MTHFR 677C→T nor 1298A→C polymorphisms were associated with memory performance. As observed for the association with CVD,[70,71] it is possible that this finding is due to lack of statistical power to detect the risk enhancement associated with the moderate increase of tHcy caused by the TT genotype.

The mechanisms behind the association among tHcy, B vitamins, and cognitive function remain unclear.[10,30,59] Currently, there is no conclusive evidence that B-vitamin deficiency is a common cause of dementia or that increased intake of B vitamins can prevent development of dementia.[30,59,72] However, proper trials on subjects with cognitive impairment have not been performed. A noteworthy finding in our study is that a decline in folate or an increase in tHcy status during the 6-year follow-up was associated with a lower KOLT score. In subjects who had a "favorable" change in folate and tHcy, the same trend, but in the opposite direction, was also observed; that is, they had a higher KOLT score. A similar result has been reported for a test of attention in the elderly, where increasing levels of tHcy over time were associated with a decline in the cognitive test score.[73] Altogether, these findings are consistent with the hypothesis that reducing tHcy levels by changes in lifestyle,[47] or by using B-vitamin supplements, may protect against memory decline in elderly people.

In conclusion, our results confirm that plasma tHcy is a strong and independent risk factor for memory impairment, with a clear dose–response relation. Our findings should motivate randomized clinical trials with tHcy-reducing therapy to provide further evidence for a relation among tHcy, B vitamins, and cognition.

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