British Journal of Nutrition (2013), **109**, 511–519
doi:10.1017/S0007114512001249

Plasma free choline, betaine and cognitive performance: the Hordaland Health Study

Eha Nurk[1,2]*, Helga Refsum[1,3], Ingvar Bjelland[4,5], Christian A. Drevon[1], Grethe S. Tell[6], Per M. Ueland[7,8], Stein E. Vollset[6,9], Knut Engedal[10], Harald A. Nygaard[6,11] and David A. Smith[3]

[1]*Department of Nutrition, Faculty of Medicine, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway*
[2]*Department of Surveillance and Evaluation, National Institute for Health Development, Hiiu 42, 11619 Tallinn, Estonia*
[3]*Department of Pharmacology, Oxford Project to Investigate Memory and Ageing (OPTIMA), University of Oxford, Oxford, UK*
[4]*Department of Child and Adolescent Psychiatry, Haukeland University Hospital, Bergen, Norway*
[5]*Department of Clinical Medicine, Faculty of Medicine and Dentistry, University of Bergen, Bergen, Norway*
[6]*Department of Public Health and Primary Health Care, University of Bergen, Bergen, Norway*
[7]*Section for Pharmacology, Institute of Medicine, Haukeland University Hospital, University of Bergen, Bergen, Norway*
[8]*Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway*
[9]*Norwegian Institute of Public Health, Bergen, Norway*
[10]*Department of Geriatric Medicine, Norwegian Centre for Dementia Research, Oslo University Hospital, Oslo, Norway*
[11]*NKS Olaviken Hospital for Old Age Psychiatry, Erdal, Norway*

(Submitted 1 September 2011 – Final revision received 23 January 2012 – Accepted 2 March 2012 – First published online 1 May 2012)

Abstract

Choline and betaine are nutrients involved in one-carbon metabolism. Choline is essential for neurodevelopment and brain function. We studied the associations between cognitive function and plasma concentrations of free choline and betaine. In a cross-sectional study, 2195 subjects (55% women), aged 70–74 years, underwent extensive cognitive testing including the Kendrick Object Learning Test (KOLT), Trail Making Test (part A, TMT-A), modified versions of the Digit Symbol Test (m-DST), Block Design (m-BD), Mini-Mental State Examination (m-MMSE) and Controlled Oral Word Association Test (COWAT). Compared with low concentrations, high choline (>8·4 μmol/l) was associated with better test scores in the TMT-A (56·0 *v.* 61·5, $P=0·004$), m-DST (10·5 *v.* 9·8, $P=0·005$) and m-MMSE (11·5 *v.* 11·4, $P=0·01$). A generalised additive regression model showed a positive dose–response relationship between the m-MMSE and choline ($P=0·012$ from a corresponding linear regression model). Betaine was associated with the KOLT, TMT-A and COWAT, but after adjustments for potential confounders, the associations lost significance. Risk ratios (RR) for poor test performance roughly tripled when low choline was combined with either low plasma vitamin B_{12} (≤257 pmol/l) concentrations ($RR_{KOLT}=2·6$, 95% CI 1·1, 6·1; $RR_{m\text{-}MMSE}=2·7$, 95% CI 1·1, 6·6; $RR_{COWAT}=3·1$, 95% CI 1·4, 7·2) or high methylmalonic acid (MMA) (≥3·95 μmol/l) concentrations ($RR_{m\text{-}BD}=2·8$, 95% CI 1·3, 6·1). Low betaine (≤31·1 μmol/l) combined with high MMA was associated with elevated RR on KOLT ($RR_{KOLT}=2·5$, 95% CI 1·0, 6·2). Low plasma free choline concentrations are associated with poor cognitive performance. There were significant interactions between low choline or betaine and low vitamin B_{12} or high MMA on cognitive performance.

Key words: **Cognitive performance: Plasma free choline: Plasma betaine**

Ageing is the most important risk factor for cognitive decline, dementia and Alzheimer's disease. Besides ageing, there are several other behavioural factors such as diet, obesity, smoking and physical activity that affect cognitive function[1]. There is considerable evidence that one-carbon metabolites are essential in neurodevelopment and brain function. High plasma concentrations of total homocysteine (tHcy) and low concentrations of folate and/or vitamin B_{12} have been

Abbreviations: COWAT, Controlled Oral Word Association Test; KOLT, Kendrick Object Learning Test; m-DST, modified version of the Digit Symbol Test; MMA, methylmalonic acid; m-MMSE, modified version of the Mini-Mental State Examination; MTHFR, methylenetetrahydrofolate reductase; RR, risk ratio; tHcy, total homocysteine; TMT-A, Trail Making Test, part A.

* **Corresponding author:** E. Nurk, fax +372 6593901, email eha.nurk@medisin.uio.no

British Journal of Nutrition

associated with cognitive deficit and Alzheimer's disease[2]. Betaine and choline are quaternary ammonium compounds metabolically linked to both lipid and folate-dependent one-carbon metabolism[3]. Choline lowers plasma tHcy concentrations, even when dietary consumption of folate and other B vitamins is adequate[4,5], and high doses of betaine, alone or in combination with other B vitamins, are used in the treatment of homocystinuria[3].

Betaine serves as a methyl donor in a reaction converting homocysteine to methionine, catalysed by the hepatic enzyme betaine-homocysteine methyltransferase[6]. It is not known whether betaine plays a role within the brain, although a betaine/γ-aminobutyric acid (GABA) transporter has been identified in astrocytes[7]. There is only one published report of a positive association between plasma concentrations of betaine and cognition in human subjects[8].

Choline, an essential dietary constituent[9], is required for the synthesis of acetylcholine, phospholipids and betaine[3]. Because acetylcholine is a neurotransmitter involved in attention, learning and memory, choline may be important in many cognitive processes and in brain development[10]. Choline, as a component of phosphatidylcholine, also plays a role in membrane structure and in membrane-mediated cell signalling[11]. In animal studies, rats in impoverished environmental conditions (no toys or other playing opportunities) fed cytidine 5'-diphosphocholine (CDP)-choline were protected from memory impairment[12]. Moreover, in rats, choline supplementation during the embryonic period improves memory performance later in life[13]. In contrast, one study found no correlation between human maternal and cord blood choline concentrations and subsequent child intelligence quotient scores at 5 years of age[14]. In a folate-fortified human population, low serum concentrations of total choline were associated with an elevated risk of neural tube defects[15]. In another study[16], a positive relationship between plasma choline concentration and acetylcholine concentration was observed in children with cystic fibrosis who had low choline status, but not in healthy children. Whether plasma choline concentrations are associated with acetylcholine concentration in older adults is not known, and controlled clinical trials have not shown any clinical value of choline and phosphatidylcholine in the treatment of cognitive dysfunction in Alzheimer's disease[17,18]. On the other hand, in a recent study in a large, non-demented community-based cohort, higher concurrent choline intake was associated with better cognitive performance[19].

Although choline plays an important role in the development as well as functioning of the central nervous system, few studies have investigated the association between plasma concentrations of choline and cognitive function. We have examined the associations between plasma concentrations of free choline, betaine and cognitive functions monitored in an elderly subsample of the Hordaland Health Study. We also investigated possible interactions with other one-carbon metabolites, because choline, betaine and folate are interchangeable sources of one-carbon units, and, together with vitamin B_{12}, determinants of tHcy[3], which is a strong marker of future cognitive decline[2].

Subjects and methods

Study population

The Hordaland Health Study was conducted from 1997 to 1999 as a collaborative effort between the University of Bergen, University of Oslo, local health services and the National Health Screening Service (now the Norwegian Institute of Public Health). Details of the study and of recruitment to the cognitive sub-study have been described elsewhere[20,21]. Briefly, the cognitive sub-study was confined to all those living in the city of Bergen and who were born between 1925 and 1927. A total of 2841 elderly subjects attended both the baseline (1992–3) and the follow-up (1997–9) studies and were in the latter study invited, independent of their cognitive status, to participate in cognitive tests; 2197 (77·3 %) of these subjects agreed to participate. In the present study, we have restricted the cross-sectional analyses to 2195 individuals for whom plasma concentrations of free choline and betaine[22] and cognitive function measurements were available. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Regional Committee for Medical Research Ethics of Western Norway. Written informed consent was obtained from all subjects.

Data collection

Cognitive testing was performed at the study location by trained nurses after the standard cardiovascular examinations of the National Health Screening Service[23] were completed. The cognitive test battery included six tests[24]: the Kendrick Object Learning Test (KOLT, episodic memory)[25]; the Trail Making Test, part A (TMT-A, sensorimotor speed)[26]; a modified version of the Digit Symbol Test (m-DST, perceptual speed and executive function)[27]; a modified form of the Block Design (m-BD) (visuospatial skills)[27]; a modified version of the Mini-Mental State Examination (m-MMSE, global cognition)[28]; an abridged version of the Controlled Oral Word Association Test (COWAT), also called 'S-task' (semantic memory)[29]. For all cognitive tests, the higher scores indicate better performance, except for the TMT-A where the speed of fulfilment is important, i.e. the shorter the time used, the better the results.

Non-fasting blood samples used for the preparation of plasma were collected into evacuated tubes containing EDTA, and stored at −80°C. Plasma concentrations of free choline, betaine, tHcy and creatinine were measured by normal-phase liquid chromatography–tandem MS detection[30]. The within- and between-day imprecision (CV) for plasma free choline and betaine varied between 2·1 and 8·8 %[30]. Plasma concentrations of folate and vitamin B_{12} were measured by *Lactobacillus casei*[31] and *L. leichmannii* microbiological assays[32]. Plasma methylmalonic acid (MMA) concentration was measured by a modified GC–MS method based on ethylchloroformate derivatisation[33]. Methylenetetrahydrofolate reductase (MTHFR) 677C → T and apoE ε4 genotypes were determined in the packed cell fraction of blood samples using the real-time and one-stage PCR techniques, respectively[34,35]. Details on self-reported information on the history of CVD,

education, smoking status and depression have been reported previously[36].

Statistical analyses

Because the distributions of blood measurements showed a markedly skewed distribution, log-transformed data were used in all analyses. Pearson's correlation coefficients were determined between choline, betaine and different covariates. Cut-off values for low plasma concentrations of free choline and betaine were set at the 20th percentile of the study population values: ≤8·4 μmol/l for choline and ≤31·1 μmol/l for betaine. For a comparison between groups, Pearson's χ^2, independent sample *t* tests and ANOVA were applied. Preliminary analyses to identify covariates showed that cognitive performance was associated with education, plasma folate concentration and depression (all test scores were significantly associated). Of the six cognitive test scores, five were significantly associated with tHcy and four were associated with apoE ε4 allele and CVD history. Sex, smoking and plasma concentration of vitamin B_{12} were significantly associated with two out of six cognitive test scores, whereas plasma concentrations of MMA and creatinine, and the MTHFR 677C → T T allele were significantly associated with only one cognitive test score. Plasma concentrations of free choline or betaine were significantly associated with sex, apoE ε4 allele, the history of CVD, education, smoking status, plasma concentrations of folate, MMA, tHcy and creatinine. Inclusion of plasma concentrations of vitamin B_{12}, MMA or tHcy as covariates did not alter the results significantly and are therefore omitted from final statistical models to avoid potential over-adjustment. Thus, the final adjusted models included the following variables: sex, education, apoE ε4 allele, CVD history, smoking status, plasma concentrations of folate and creatinine, and MTHFR 677C → T genotype. Although the depression score was highly correlated with cognitive performance, it was excluded from statistical models due to a large proportion of missing values. However, the effect of depression is reported separately when it affected otherwise significant results. Given the narrow age range, adjustment for age did not change the results and has not been included. In multivariate analyses, subjects with missing data in one or more variables were excluded. Gaussian generalised additive regression models, as implemented in S-PLUS 6.2 for Windows (Insightful Corporation), were used to generate graphic representations of the dose–response relationships, using a sex-adjusted model. Multiple linear regression analyses were used to examine significant associations between the cognitive test scores and plasma concentrations of free choline and betaine using both a sex-adjusted model and a model adjusted for the variables referred to the final model above. Potential interaction between plasma free choline or betaine and other one-carbon metabolites (plasma concentrations of folate, vitamin B_{12}, MMA and tHcy) on poor cognitive performance (cut-off points for poor cognitive test scores were set at about the 10th percentile of the cognitive test score, except for the TMT-A, for which the 90th percentile was used) was assessed by multiple logistic regression analyses including an interaction term where plasma free choline and betaine concentrations were dichotomous variables both as a main effect and an interaction term. Similarly, other one-carbon metabolites were present in interaction models as dichotomous variables. Cut-off values for low or high concentrations were set either at the 20th or 80th percentile: low folate ≤5·06 nmol/l; low vitamin B_{12} ≤257 pmol/l; high tHcy ≥14·3 μmol/l; high MMA ≥3·95 μmol/l. All interaction models were adjusted for the same covariates as mentioned for the final models above, except for the models where plasma concentration of folate was studied as an effect modifier.

All statistical analyses, except for generalised additive models, were performed using the SPSS version 16.0 for Windows (SPSS, Inc.). A two-sided *P* value less than 0·05 was considered significant.

Results

The characteristics of the study population, including cognitive scores, are presented in Table 1. Of the 1992 participants who completed all cognitive tests, 231 (less than 12 %) performed poorly in two or more tests, including four individuals who

Table 1. Characteristics of the study population

(Number of participants and percentages; mean values and 95 % confidence intervals)

	Total *n*	Mean*	95 % CI
Sex			
Male (%)	2195	45	
MTHFR 677C → T, T allele frequency (%)	2195	49·8	
ApoE ε4 allele frequency (%)	2180	32·1	
Depression, HADS score ≥8 (%)	1883	9·2	
Previous CVD (%)	2046	35·3	
Education			
≤ 9 years (%)	2157	40·9	
Smoking			
Current smokers (%)	2195	43·1	
Blood parameters			
Choline (μmol/l)	2195	10·1	10·0, 10·2
Betaine (μmol/l)	2195	39·3	38·8, 39·7
Folate (nmol/l)	2184	7·5	7·4, 7·7
Vitamin B_{12} (pmol/l)	2192	348	342, 354
Methylmalonic acid (μmol/l)	2192	0·20	0·20, 0·20
Total homocysteine (μmol/l)	2195	11·6	11·4, 11·7
Creatinine (μmol/l)	2195	91·7	91·1, 92·3
Cognitive performance			
KOLT	2189	35·1	34·8, 35·5
TMT-A	2185	57·6	56·2, 59·1
m-DST	2180	10·2	10·1, 10·4
m-BD	2178	15·0	14·9, 15·1
m-MMSE	2173	11·5	11·5, 11·5
COWAT	2185	15·1	14·8, 15·3

MTHFR, methylenetetrahydrofolate reductase; HADS, Hospital Anxiety and Depression Scale; KOLT, Kendrick Object Learning Test (episodic memory); TMT-A, part A of the Trail Making Test (sensorimotor speed); m-DST, modified version of the Digit Symbol Test (perceptual speed and executive function); m-BD, modified version of the Block Design (visuospatial skills); m-MMSE, modified version of the Mini-Mental State Examination (global cognition); COWAT, S-task from the Controlled Oral Word Association Test (semantic memory).

*Geometric means for blood parameters.

British Journal of Nutrition

performed poorly in all six tests, nine in five tests, thirty in four tests, sixty-five in three tests and 123 in two tests.

The correlation coefficients between choline, betaine and other plasma values, genetic factors, lifestyle variables and cognitive performance are presented in Table 2. The strongest correlation was between plasma concentrations of choline and betaine, but also several other blood parameters, notably plasma creatinine, correlated with choline and betaine. CVD history and education were correlated with choline as well as betaine, and betaine was also correlated with the number of apoE ε4 alleles. Episodic memory was negatively and sensorimotor speed positively correlated with plasma concentrations of choline as well as betaine, although these correlations were weak. Global cognition was positively correlated with plasma choline concentration, and perceptual speed, executive function and semantic memory were positively correlated with plasma betaine concentration.

Men had significantly higher mean plasma concentrations of both choline and betaine than women: 10·8 (95 % CI 10·6, 10·9) *v.* 9·6 (95 % CI 9·5, 9·7) μmol/l ($P<0·001$) for choline and 43·9 (95 % CI 43·2, 44·6) *v.* 35·9 (95 % CI 35·3, 36·4) μmol/l ($P<0·001$) for betaine. Participants with low plasma concentrations of choline compared with those with high concentrations had lower concentrations of betaine (mean 33·0 (95 % CI 32·1, 33·9) *v.* 41·0 (95 % CI 40·5, 41·5) μmol/l, $P<0·001$), MMA (mean 0·19 (95 % CI 0·18, 0·20) *v.* 0·20 (95 % CI 0·20, 0·21) μmol/l, $P<0·001$) and creatinine (mean 87 (95 % CI 85, 88) *v.* 93 (95 % CI 92, 94) μmol/l, $P<0·001$). Compared with high betaine status, subjects with low plasma concentrations of betaine had lower concentrations of choline (mean 8·7 (95 % CI 8·5, 8·9) *v.* 10·5 (95 % CI 10·4, 10·6) μmol/l, $P<0·001$), folate (mean 6·8 (95 % CI 6·5, 7·2) *v.* 7·7 (95 % CI 7·6, 8·0) μmol/l, $P<0·001$), MMA (mean 0·19 (95 % CI 0·19, 0·20) *v.* 0·20 (95 % CI 0·20, 0·21) μmol/l, $P=0·025$) and creatinine (mean 88 (95 % CI 87, 89) *v.* 93 (95 % CI 92, 93) μmol/l, $P<0·001$), but higher concentrations of tHcy (mean 12·4 (95 % CI 12·0, 12·8) *v.* 11·4 (95 % CI 11·2, 11·5) μmol/l, $P<0·001$).

Participants with low plasma choline concentrations had poorer cognitive performance in sensorimotor speed, perceptual speed and executive function than subjects with high plasma free choline concentrations (Table 3); the results became more significant for sensorimotor speed, perceptual speed and executive function, and global cognition after multiple adjustments for sex, education, apoE ε4 allele, CVD history, smoking status, plasma concentrations of folate and creatinine, and MTHFR 677C → T genotype. Participants with low plasma betaine concentrations performed significantly better than those with high betaine concentrations in episodic memory, whereas performance related to sensorimotor speed was worse (Table 4). After multiple adjustments, the betaine associations were no longer significant.

There was a positive dose–response relationship between global cognition (m-MMSE score) and plasma choline concentrations (Fig. 1), and the association remained significant in the linear regression model after adjustments for sex, education, apoE ε4 allele, CVD history, smoking status, plasma concentrations of folate and creatinine, and MTHFR 677C → T genotype ($P=0·012$). Plasma betaine concentration was positively

Table 2. Pearson's correlations between plasma concentrations of free choline, betaine and different covariates (Number of participants and correlation coefficients)

		Choline		Betaine	
	n	Correlation coefficient	*P*	Correlation coefficient	*P*
Sex					
Female	2195	−0·247	<0·001	−0·349	<0·001
MTHFR 677C → T	2195	−0·032	0·14	−0·019	0·37
ApoE ε4 allele	2180	0·033	0·12	0·045	0·034
Depression	1883	−0·023	0·32	−0·016	0·49
Previous CVD	2046	0·102	<0·001	0·065	0·003
Education	2157	0·055	0·011	0·122	<0·001
Smoking status	2195	0·004	0·84	0·014	0·52
Plasma measurements					
Betaine (μmol/l)	2195	0·439	<0·001		
Folate (nmol/l)	2184	0·060	0·005	0·122	<0·001
Vitamin B_{12} (pmol/l)	2192	−0·036	0·09	0·000	0·97
Methylmalonic acid (μmol/l)	2192	0·079	<0·001	0·047	0·027
Total homocysteine (μmol/l)	2195	0·056	0·008	−0·162	<0·001
Creatinine (μmol/l)	2195	0·294	<0·001	0·150	<0·001
Cognitive performance					
KOLT	2189	−0·043	0·046	−0·063	0·003
TMT-A	2185	−0·043	0·042	−0·065	0·002
m-DST	2180	0·022	0·31	0·042	0·049
m-BD	2178	0·009	0·68	0·021	0·34
m-MMSE	2173	0·044	0·042	0·038	0·08
COWAT	2185	0·011	0·62	0·043	0·045

MTHFR, methylenetetrahydrofolate reductase; KOLT, Kendrick Object Learning Test (episodic memory); TMT-A, part A of the Trail Making Test (sensorimotor speed); m-DST, modified version of the Digit Symbol Test (perceptual speed and executive function); m-BD, modified version of the Block Design (visuospatial skills); m-MMSE, modified version of the Mini-Mental State Examination (global cognition); COWAT, S-task from the Controlled Oral Word Association Test (semantic memory).

British Journal of Nutrition

Table 3. Cognitive test performance by status of plasma concentration of free choline*
(Mean values and 95 % confidence intervals, number of participants and percentages)

	≤8·36 μmol/l				>8·36 μmol/l					
	n	Mean	95 % CI	%	*n*	Mean	95 % CI	%	*P*†	*P*‡
KOLT										
Score	435	35·5	34·7, 36·3		1754	35·2	34·8, 35·5		0·85	0·58
Poor performance	47			10·8	188			10·7	0·96	
TMT-A										
Score	434	61·9	58·3, 65·5		1751	56·2	54·6, 57·8		0·040	0·004
Poor performance	57			13·1	169			9·7	0·033	
m-DST										
Score	432	9·7	9·3, 10·1		1748	10·4	10·2, 10·6		0·023	0·005
Poor performance	43			10·0	151			8·6	0·39	
m-BD										
Score	430	15·0	14·7, 15·2		1748	15·0	14·9, 15·1		0·84	0·83
Poor performance	70			16·3	253			14·5	0·35	
m-MMSE										
Score	428	11·4	11·4, 11·5		1745	11·5	11·5, 11·9		0·062	0·010
Poor performance	51			11·9	160			9·2	0·09	
COWAT										
Score	433	14·9	14·4, 15·4		1752	15·1	14·9, 15·4		0·22	0·25
Poor performance	53			12·2	186			10·6	0·33	

KOLT, Kendrick Object Learning Test (episodic memory); TMT-A, part A of the Trail Making Test (sensorimotor speed); m-DST, modified version of the Digit Symbol Test (perceptual speed and executive function); m-BD, modified version of the Block Design (visuospatial skills); m-MMSE, modified version of the Mini-Mental State Examination (global cognition); COWAT, S-task from the Controlled Oral Word Association Test (semantic memory).

* Cut-off value for the low plasma concentration of free choline was set at the 20th percentile of the study population values. Cut-off points for poor cognitive test performance were set at about the 10th percentile of the cognitive test score, except for the TMT-A, for which the 90th percentile was used: KOLT ≤25; TMT-A ≥111; m-DST ≤5; m-BD ≤12; m-MMSE ≤10; COWAT ≤8.

† Independent sample *t* test or Pearson's χ^2.

‡ Univariate ANOVA, adjusted for sex, education, apoE ε4 allele, CVD history, smoking status, plasma concentrations of folate and creatinine and methylenetetrahydrofolate reductase 677C → T genotype.

and linearly associated with sensorimotor speed (TMT-A score) and semantic memory (COWAT score), and there were also borderline significant linear associations with global cognition (m-MMSE score), perceptual speed and executive function (m-DST score). However, none of the linear associations between plasma concentrations of betaine and cognitive test performances remained significant after multiple adjustments.

The interaction analyses based on cross-sectional data showed that low plasma free choline concentrations combined either with low vitamin B_{12} or high plasma MMA concentration increased the risk ratio (RR) for poor performance by 2·6–3·1-fold in episodic memory, global cognition, semantic memory and visuospatial skills (Table 5). Similarly, low plasma concentrations of betaine combined with high plasma concentrations of MMA increased the RR for poor performance in episodic memory 2·5 times (Table 5). In addition, we found that low plasma concentrations of free choline and betaine together more than doubled the RR for poor visuospatial skills, the RR being 2·01 (95 % CI 0·98, 4·12, P=0·056). There were no significant interactions between low plasma concentrations of free choline or betaine and low folate or high tHcy concentrations on cognitive functions (data not shown).

Because inclusion of the depression score as a covariate in the interaction models significantly reduced the number of participants due to missing data, it was excluded from the final models. However, although most of the interactions maintained their strength and remained significant after adjusting for depression (data not shown), the RR were no longer significant for combinations of low choline and low vitamin B_{12} on the KOLT (RR 2·30, 95 % CI 0·88, 6·03) and m-MMSE scores (RR 2·41, 95 % CI 0·90, 6·46).

Discussion

In a population-based elderly cohort of 2195 individuals, we have shown that low plasma concentrations of free choline were cross-sectionally associated with poor performance in global cognition, sensorimotor speed, perceptual speed and executive function, after adjusting for other factors known to influence cognition. The associations between plasma betaine concentrations and cognitive function were no longer significant after controlling for these other risk factors. There were significant interactions between low plasma concentrations of free choline or betaine and markers of vitamin B_{12} status (plasma vitamin B_{12} and MMA) on cognitive performance.

The associations of plasma or serum concentrations of choline and betaine with cognition in human subjects have rarely been studied. Among Dutch elderly people, plasma betaine concentrations were positively associated with the domains of construction, sensorimotor speed and executive function[8]. In addition, there was a tendency that participants with the largest increase in betaine concentrations showed a larger increase in memory performance when compared with participants with the smallest increase in betaine concentrations after 24 weeks of supplementation with folate and vitaminie: B_{12}[8]. In line with those results, we found positive cross-sectional associations between plasma concentrations of betaine and sensorimotor speed, executive function, perceptual speed and

British Journal of Nutrition

Table 4. Cognitive test performance by status of plasma concentration of betaine*
(Mean values and 95 % confidence intervals, number of participants and percentages)

	≤31·1 μmol/l				>31·1 μmol/l					
	n	Mean	95 % CI	%	*n*	Mean	95 % CI	%	*P*†	*P*‡
KOLT										
Score	440	36·4	35·6, 37·2		1749	34·9	34·6, 35·3		<0·001	0·13
Poor performance	38			8·6	197			11·3	0·11	
TMT-A										
Score	440	60·7	57·3, 64·0		1745	56·5	54·9, 58·1		0·034	0·88
Poor performance	41			9·3	185			10·6	0·43	
m-DST										
Score	439	10·1	9·7, 10·5		1741	10·3	10·1, 10·5		0·58	0·71
Poor performance	40			9·1	154			8·8	0·85	
m-BD										
Score	439	15·0	14·7, 15·2		1739	15·0	14·9, 15·1		0·39	0·82
Poor performance	68			15·5	255			14·7	0·66	
m-MMSE										
Score	438	11·5	11·5, 11·6		1735	11·5	11·5, 11·6		0·64	0·58
Poor performance	37			8·4	174			10·0	0·32	
COWAT										
Score	440	14·9	14·4, 15·4		1745	15·1	14·9, 15·4		0·44	0·92
Poor performance	47			10·7	192			11·0	0·93	

KOLT, Kendrick Object Learning Test (episodic memory); TMT-A, part A of the Trail Making Test (sensorimotor speed); m-DST, modified version of the Digit Symbol Test (perceptual speed and executive function); m-BD, modified version of the Block Design (visuospatial skills); m-MMSE, modified version of the Mini-Mental State Examination (global cognition); COWAT, S-task from the Controlled Oral Word Association Test (semantic memory).
* Cut-off value for the low plasma concentration of betaine was set at the 20th percentile of the study population values. Cut-off points for poor cognitive test performance were set at about the 10th percentile of the cognitive test score, except for the TMT-A, for which the 90th percentile was used: KOLT ≤25; TMT-A ≥111; m-DST ≤5; m-BD ≤12; m-MMSE ≤10; COWAT ≤8.
† Independent sample *t* test or Pearson's χ^2.
‡ Univariate ANOVA, adjusted for sex, education, apoE ε4 allele, CVD history, smoking status, plasma concentrations of folate and creatinine and methylenetetrahydrofolate reductase 677C → T genotype.

Table 5. Interaction between low plasma concentrations of free choline or betaine and different covariates on poor cognitive performance*
(Risk ratios and 95 % confidence intervals)

	Risk ratio†	95 % CI	*P*	Risk ratio†‡	95 % CI‡	*P*‡
KOLT						
Low choline	1·01	0·72, 1·42	0·95	1·16	0·79, 1·68	0·45
Low vitamin B_{12}	1·01	0·72, 1·41	0·97	0·96	0·66, 1·38	0·82
Low choline and vitamin B_{12}	2·88	1·32, 6·28	0·008	2·57	1·09, 6·06	0·030
KOLT						
Low betaine	0·75	0·52, 1·08	0·13	0·98	0·65, 1·48	0·94
High MMA	1·24	0·90, 1·71	0·19	1·19	0·84, 1·71	0·33
Low betaine and high MMA	2·17	0·94, 5·00	0·07	2·53	1·03, 6·21	0·043
m-BD						
Low choline	1·16	0·87, 1·54	0·33	1·10	0·79, 1·51	0·58
High MMA	1·07	0·80, 1·43	0·67	1·11	0·81, 1·54	0·51
Low choline and high MMA	2·13	1·04, 4·37	0·038	2·80	1·28, 6·14	0·010
m-MMSE						
Low choline	1·31	0·94, 1·84	0·11	1·38	0·95, 2·01	0·10
Low vitamin B_{12}	1·04	0·73, 1·48	0·84	1·04	0·70, 1·54	0·86
Low choline and vitamin B_{12}	2·95	1·35, 6·45	0·007	2·72	1·13, 6·56	0·025
COWAT						
Low choline	1·18	0·85, 1·63	0·33	1·40	0·97, 2·01	0·07
Low vitamin B_{12}	1·02	0·73, 1·43	0·89	1·13	0·78, 1·63	0·53
Low choline and vitamin B_{12}	3·06	1·44, 6·51	0·004	3·13	1·37, 7·18	0·007

KOLT, Kendrick Object Learning Test (episodic memory); MMA, methylmalonic acid; m-BD, modified version of the Block Design (visuospatial skills); m-MMSE, modified version of the Mini-Mental State Examination (global cognition); COWAT, S-task from the Controlled Oral Word Association Test (semantic memory).
* Cut-off values for low choline and low or high concentrations of other variables were set either at 20th or 80th percentile: low choline ≤8·36 μmol/l; low betaine ≤31·1 μmol/l; low vitamin B_{12} ≤257 pmol/l; high MMA ≥3·95 μmol/l. Cut-off points for poor cognitive test scores were set at about the 10th percentile of the cognitive test score: KOLT ≤25; m-BD ≤12; m-MMSE ≤10; COWAT ≤8.
† The reference group includes subjects with normal concentrations of both plasma free choline or betaine and vitamin B_{12} or MMA.
‡ Adjusted for sex, education, apoE ε4 allele, history of CVD, smoking status, plasma folate, creatinine and methylenetetrahydrofolate reductase 677C → T genotype.

British Journal of Nutrition

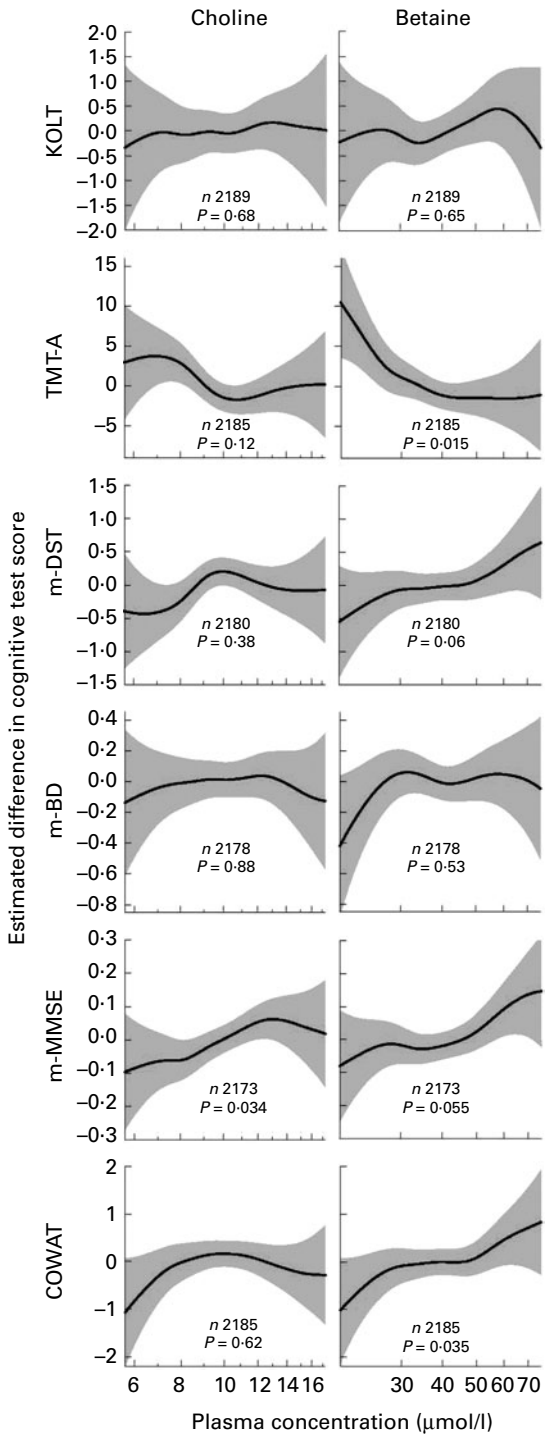

Fig. 1. Associations between different cognitive test scores and plasma concentrations of choline and betaine obtained by Gaussian generalised additive regression models. On the vertical axis, the model generates a reference value of zero that approximately corresponds to the value of cognitive test score associated with the mean of plasma concentrations of choline and betaine for all subjects. Solid lines are the estimated dose–response curves; shaded areas represent 95 % CI. *P* values adjusted for sex are from corresponding multiple linear regression analyses. The data for the lowest and highest 1 percentile of plasma concentrations are not included. KOLT, Kendrick Object Learning Test; TMT-A, part A of the Trail Making Test; m-DST, modified version of the Digit Symbol Test; m-BD, modified version of the Block Design; m-MMSE, modified version of the Mini-Mental State Examination; COWAT, abridged version of the Controlled Oral Word Association Test (S-task).

semantic memory. However, the associations disappeared after multiple adjustments. Eussen *et al.*[8] did not find significant associations between cognition and plasma choline concentration. In contrast, we observed that choline was positively associated with sensorimotor speed, perceptual speed, executive function and global cognition. Moreover, we also observed a significant dose–response relationship between plasma concentrations of choline and global cognition. The reasons for the differences in observations between the present findings and those of Eussen *et al.*[8] are unknown, but may be partly due to different study designs and sample sizes, as the Dutch study was a randomised, double-blind, placebo-controlled trial with 195 participants.

Surprisingly, episodic memory was inversely correlated with plasma concentrations of choline and betaine in our dataset. The mechanisms behind these associations are unclear, or as the associations were relatively weak and there was no dose–response effect, these associations may appear by chance.

It has been suggested that plasma concentration of free choline represents only a minor fraction of the total choline pool, and thus may be a poor marker of choline status and metabolism in the brain[8]. Moreover, even if administration of free choline increases brain choline availability, it does not increase acetylcholine synthesis or release, which may explain its ineffectiveness in relieving the cognitive symptoms of Alzheimer's disease[17]. In animal studies, betaine concentration among different tissues was lowest in the brain and was about 25 % of that in the plasma; and there was no relationship between brain and plasma betaine concentrations[37]. These findings may explain why the associations between cognition and plasma concentrations of free choline or betaine in different studies are inconsistent and indicate that plasma free choline and betaine themselves are inadequate predictors of status of these nutrients in tissues.

The effects of betaine and choline on one-carbon metabolism are often present only in subgroups, for example, among subjects with folate deficiency or with low plasma concentrations of other B vitamins (B_2, B_6 and B_{12}) in combination with the TT genotype of the MTHFR 677C → T polymorphism[3]. This reflects the convergence of both betaine-homocysteine methyltransferase and vitamin B_{12}-dependent methionine synthase on methionine formation, whereby folate, choline and betaine become fungible sources of one-carbon units[3]. These interrelationships may explain why combined abnormal concentrations of one-carbon metabolites have a stronger effect on cognition than each metabolite alone. In the present study, neither low concentrations of vitamin B_{12}, nor high concentrations of MMA alone, were associated with poor cognitive performance, but low vitamin B_{12} concentration in combination with low plasma concentration of choline nearly tripled the RR for poor cognitive performance related to episodic memory, global cognition and semantic memory. Similarly, high MMA concentration combined with low betaine or low plasma free choline concentrations more than doubled the RR for poor performance in episodic memory and visuospatial skills, respectively.

The strengths of the present study include a large population-based sample with six different cognitive tests. A major

limitation of the study is the cross-sectional design as cognition in the elderly results from long-term exposures[38,39] and subjects with impaired cognition may have altered their diet as a consequence of a change in their cognitive function. However, because the cognition of participants in the present study was not seriously impaired, we do not believe that the present findings are related to reversed causality. There is also a risk for type I error as the significant associations in the present study are often weak and partly contrasting. Last but not least, as 77·3 % of the 2841 study attendees volunteered for cognitive testing, the possibility of recruitment bias should be considered.

In conclusion, the overall associations between cognition and plasma concentrations of free choline and betaine in human subjects seem to be modest and further investigations are needed, particularly in relation to interactions with other risk factors, and with genetic polymorphisms that may affect choline[40].

Acknowledgements

The present study was supported by the Charles Wolfson Charitable Trust, Alzheimer's Research (UK), the Advanced Research Programme of Norway, the Johan Throne Holst Foundation for Nutrition Research, the Freia Medical Foundation, University of Oslo, and the Foundation to Promote Research into Functional Vitamin B_{12} Deficiency (Norway). We are grateful to Elfrid Blomdal (University of Bergen, Norway) for her excellent support with the literature. H. R., G. S. T., P. M. U. and S. E. V. participated in the study design and the organisation of the data collection. K. E., H. A. N. and A. D. S. assisted with the design and organisation of the cognitive sub-study. E. N. conducted the statistical analyses and wrote the first draft of the manuscript. All co-authors interpreted the results, contributed to the study design and participated in critically revising the manuscript. None of the authors had any conflict of interest.

References

1. Lee Y, Back JH, Kim J, *et al.* (2010) Systematic review of health behavioral risks and cognitive health in older adults. *Int Psychogeriatr* **22**, 174–187.
2. Smith AD (2008) The worldwide challenge of the dementias: a role for B vitamins and homocysteine? *Food Nutr Bull* **29**, Suppl. 2, S143–S172.
3. Ueland PM (2010) Choline and betaine in health and disease. *J Inherit Metab Dis* **34**, 3–15.
4. da Costa KA, Gaffney CE, Fischer LM, *et al.* (2005) Choline deficiency in mice and humans is associated with increased plasma homocysteine concentration after a methionine load. *Am J Clin Nutr* **81**, 440–444.
5. Lee JE, Jacques PF, Dougherty L, *et al.* (2010) Are dietary choline and betaine intakes determinants of total homocysteine concentration? *Am J Clin Nutr* **91**, 1303–1310.
6. Ueland PM, Holm PI & Hustad S (2005) Betaine: a key modulator of one-carbon metabolism and homocysteine status. *Clin Chem Lab Med* **43**, 1069–1075.
7. Madsen KK, White HS & Schousboe A (2010) Neuronal and non-neuronal GABA transporters as targets for antiepileptic drugs. *Pharmacol Ther* **125**, 394–401.
8. Eussen SJ, Ueland PM, Clarke R, *et al.* (2007) The association of betaine, homocysteine and related metabolites with cognitive function in Dutch elderly people. *Br J Nutr* **98**, 960–968.
9. Zeisel SH & da Costa KA (2009) Choline: an essential nutrient for public health. *Nutr Rev* **67**, 615–623.
10. Zeisel SH (2004) Nutritional importance of choline for brain development. *J Am Coll Nutr* **23**, Suppl. 6, 621S–626S.
11. Zeisel SH (2006) The fetal origins of memory: the role of dietary choline in optimal brain development. *J Pediatr* **149**, Suppl. 5, S131–S136.
12. Teather LA & Wurtman RJ (2005) Dietary CDP-choline supplementation prevents memory impairment caused by impoverished environmental conditions in rats. *Learn Mem* **12**, 39–43.
13. McCann JC, Hudes M & Ames BN (2006) An overview of evidence for a causal relationship between dietary availability of choline during development and cognitive function in offspring. *Neurosci Biobehav Rev* **30**, 696–712.
14. Signore C, Ueland PM, Troendle J, *et al.* (2008) Choline concentrations in human maternal and cord blood and intelligence at 5 y of age. *Am J Clin Nutr* **87**, 896–902.
15. Shaw GM, Finnell RH, Blom HJ, *et al.* (2009) Choline and risk of neural tube defects in a folate-fortified population. *Epidemiology* **20**, 714–719.
16. Innis SM, Davidson AGF, Bay BN, *et al.* (2011) Plasma choline depletion is associated with decreased peripheral blood leukocyte acetylcholine in children with cystic fibrosis. *Am J Clin Nutr* **93**, 564–568.
17. Amenta F & Tayebati SK (2008) Pathways of acetylcholine synthesis, transport and release as targets for treatment of adult-onset cognitive dysfunction. *Curr Med Chem* **15**, 488–498.
18. Higgins JPT & Flicker L (2009) Lecithin for dementia and cognitive impairment. *Cochrane Database of Systematic Reviews 2000*, Issue 4, article no. CD001015. http://www2.cochrane.org/reviews/en/ab001015.html.
19. Poly C, Massaro JM, Seshadri S, *et al.* (2011) The relation of dietary choline to cognitive performance and white-matter hyperintensity in the Framingham Offspring Cohort. *Am J Clin Nutr* **94**, 1584–1591.
20. Refsum H, Nurk E, Smith AD, *et al.* (2006) The Hordaland Homocysteine Study: a community-based study of homocysteine, its determinants, and associations with disease. *J Nutr* **136**, Suppl. 6, 1731S–1740S.
21. Recruitment into the Cognitive Sub-study of the Hordaland Homocysteine Study. http://www.uib.no/isf/husk, www.uib.no/isf/husk/Vedlegg_dokumenter/Cognitive_Sub_study.pdf (accessed June 2011 (cited 15 September 2010)).
22. Konstantinova SV, Tell GS, Vollset SE, *et al.* (2008) Divergent associations of plasma choline and betaine with components of metabolic syndrome in middle age and elderly men and women. *J Nutr* **138**, 914–920.
23. Bjartveit K, Foss OP, Gjervig T, *et al.* (1979) The Cardiovascular Disease Study in Norwegian counties: background and organization. *Acta Med Scand* **634**, Suppl., 1–70.
24. Nurk E, Drevon CA, Refsum H, *et al.* (2007) Cognitive performance among the elderly and dietary fish intake: the Hordaland Health Study. *Am J Clin Nutr* **86**, 1470–1478.
25. Kendrick DC (1985) *Kendrick Cognitive Tests for the Elderly*. Windsor: The NFER-NELSON Publishing Company Ltd.
26. Reitan RM (1958) Validity of the trail making test as an indicator of organic brain damage. *Percept Mot Skills* **8**, 271–276.
27. Wechsler D (1981) *Wechsler Adult Intelligence Scale-Revised*. New York, NY: The Psychological Corporation.

British Journal of Nutrition

28. Braekhus A, Laake K & Engedal K (1992) The Mini-Mental State Examination: identifying the most efficient variables for detecting cognitive impairment in the elderly. *J Am Geriatr Soc* **40**, 1139–1145.
29. Benton A & Hamsher K (1989) *Multilingual Aphasia Examination*. Iowa: AJA Associates.
30. Holm PI, Ueland PM, Kvalheim G, *et al.* (2003) Determination of choline, betaine, and dimethylglycine in plasma by a high-throughput method based on normal-phase chromatography–tandem mass spectrometry. *Clin Chem* **49**, 286–294.
31. Molloy AM & Scott JM (1997) Microbiological assay for serum, plasma, and red cell folate using cryopreserved, microtiter plate method. *Methods Enzymol* **281**, 43–53.
32. Kelleher BP & Broin SD (1991) Microbiological assay for vitamin B_{12} performed in 96-well microtitre plates. *J Clin Pathol* **44**, 592–595.
33. Husek P (1995) Simultaneous profile analysis of plasma amino and organic acids by capillary gas chromatography. *J Chromatogr B Biomed Appl* **669**, 352–357.
34. Ulvik A & Ueland PM (2001) Single nucleotide polymorphism (SNP) genotyping in unprocessed whole blood and serum by real-time PCR: application to SNPs affecting homocysteine and folate metabolism. *Clin Chem* **47**, 2050–2053.
35. Wenham PR, Price WH & Blandell G (1991) Apolipoprotein E genotyping by one-stage PCR. *Lancet* **337**, 1158–1159.
36. Nurk E, Refsum H, Drevon CA, *et al.* (2010) Cognitive performance among the elderly in relation to the intake of plant foods. The Hordaland Health Study. *Br J Nutr* **104**, 1190–1201.
37. Slow S, Lever M, Chambers ST, *et al.* (2009) Plasma dependent and independent accumulation of betaine in male and female rat tissues. *Physiol Res* **58**, 403–410.
38. Launer LJ (2005) The epidemiologic study of dementia: a life-long quest? *Neurobiol Aging* **26**, 335–340.
39. Whalley LJ, Dick FD & McNeill G (2006) A life-course approach to the aetiology of late-onset dementias. *Lancet Neurol* **5**, 87–96.
40. Zeisel SH (2011) Nutritional genomics: defining the dietary requirement and effects of choline. *J Nutr* **141**, 531–534.

British Journal of Nutrition