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Effect of 3-year folic acid supplementation on cognitive function in older adults in the FACIT trial: a randomised, double blind, controlled trial

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Summary

Background **Low folate and raised homocysteine concentrations in blood are associated with poor cognitive performance in the general population. As part of the FACIT trial to assess the effect of folic acid on markers of atherosclerosis in men and women aged 50–70 years with raised plasma total homocysteine and normal serum vitamin B_{12} at screening, we report here the findings for the secondary endpoint: the effect of folic acid supplementation on cognitive performance.**

Methods **Our randomised, double blind, placebo controlled study took place between November, 1999, and December, 2004, in the Netherlands. We randomly assigned 818 participants 800 µg daily oral folic acid or placebo for 3 years. The effect on cognitive performance was measured as the difference between the two groups in the 3-year change in performance for memory, sensorimotor speed, complex speed, information processing speed, and word fluency. Analysis was by intention-to-treat. This trial is registered with clinicaltrials.gov with trial number NCT00110604.**

Findings **Serum folate concentrations increased by 576% (95% CI 539 to 614) and plasma total homocysteine concentrations decreased by 26% (24 to 28) in participants taking folic acid compared with those taking placebo. The 3-year change in memory (difference in *Z* scores 0·132, 95% CI 0·032 to 0·233), information processing speed (0·087, 0·016 to 0·158) and sensorimotor speed (0·064, –0·001 to 0·129) were significantly better in the folic acid group than in the placebo group.**

Interpretation **Folic acid supplementation for 3 years significantly improved domains of cognitive function that tend to decline with age.**

Introduction

Cognitive function declines with ageing, especially cognitive domains related to memory and information processing speed.[1] Changes in cognitive performance, especially memory function, have been linked to risk of dementia in old age.[2,3] Modifiable risk factors for age-related cognitive decline have been identified, but their causality has not yet been established.[4] Poor folate status is one such suspected risk factor.[5,6]

A longitudinal study,[7] undertaken in the USA when folic acid fortification of foods was routine, showed greater cognitive decline in people with a high folic acid intake than in those with low intakes. A systematic review of supplementation with folic acid alone or in combination with other B vitamins showed that no beneficial effect on cognitive performance was conferred.[8] Many of the trials have used small study populations, supplemented for a short duration, or used tests such as the Mini-Mental State Examination,[9] which are unable to detect subtle changes in cognitive function (table 1).[10–19]

We investigated whether 800 µg daily oral folic acid supplementation for 3 years improved cognitive performance compared with placebo in older adults. Cognitive performance was assessed with tests that probe cognitive domains that decline in the ageing process.

Methods

Participants

Participants were men and post-menopausal women aged 50–70 years, from the Gelderland region in the Netherlands who participated in the Folic Acid and Carotid Intima-media Thickness (FACIT) trial (unpublished), a study investigating the effect of folic acid supplementation on atherosclerotic progression. Additional outcomes of the trial were age-related decline in cognitive function and hearing. Here we present data for the effect of folic acid on the cognitive performance aspect of the study.

We used municipal and blood-bank registries to recruit participants. On the assumption that high concentrations of plasma total homocysteine were a risk factor for vascular disease, we selected participants expected to benefit from folic acid's homocysteine-lowering effect and excluded participants with concentrations of plasma total homocysteine of less than 13 µmol/L (73% of those screened). We excluded participants with raised homocysteine concentrations (>26 µmol/L) that were possibly due to factors other than suboptimal folate concentrations, including: serum vitamin B_{12} concentration of less than 200 pmol/L (10% of those screened); self-reported medical diagnosis of renal or thyroid disease; or self-reported use of medications that influence folate metabolism.[20] Additionally, we excluded participants with self-reported intestinal disease and participants who

reportedly used B-vitamin supplements or drugs that could affect atherosclerotic progression. Finally, more than 80% self-reported compliance during a 6-week placebo run-in period was required. The Wageningen University medical ethics committee approved the study and participants gave written informed consent.

Procedures

After the initial measurement sessions, participants were allocated placebo or 800 µg per day folic acid, which is regarded as a low dose for a clinical trial. Patients were allocated treatment or placebo with permuted blocks of sizes four and six, which varied randomly. Specialised staff who were not involved in the study allocated and labelled the capsule boxes with participants' unique sequence number. Participants in the same household received the same treatment. The folic acid and placebo capsules, produced by Swiss-Caps Benelux (Heerhugowaard, Netherlands), were indistinguishable in appearance. Capsules were individually packaged in foil strips containing 28 capsules per strip, with the days of the week printed on the back. Every year, participants received a 13-month supply of capsules. Compliance was judged by capsule-return counts and a diary that registered missed capsules. Diaries and capsules were returned by participants every 12 weeks.

At the end of the study, the proportion of participants who thought they had received folic acid or placebo did not differ between the two groups (p=0·64). 70% of participants in the folic acid group and 71% in the placebo group thought they had been allocated folic acid; whereas 11% in the folic acid group and 9% in the placebo group thought

	Duration	Number at follow-up (in analyses)	Mean age, years, SD)	Population type	Dose of folic acid (dose of placebo), mg per day	Types of cognitive test	Conclusion
Folic acid only							
Fioravanti et al[10]	60 days	29	80 (6)	Patients with memory complaints, Mini-Mental State Examination score between 16 and 24, mild to moderate cognitive decline on basis of Global Deterioration Score, 70–90 years, serum folate <7 nmol/L	15	1 Randt Memory Test a Acquistion and recall b Delayed recall c Memory index d Encoding factor e Cognitive efficiency f Attention efficiency	Folic acid improved attention efficiency score (p<0·05). When taking into account baseline folate status, folic acid improved acquisition and recall (p<0·007), delayed recall (p<0·007), memory index (p<0.002), encoding (p<0·005).
Sommer et al[11]	10 weeks	7	77 (4)	Patients meeting DSM-III-R criteria for dementia, ≥65 years, suboptimal folate (serum folate 2–5 ng/mL, red-blood-cell folate 127–452 ng/mL), B12 >200 pg/mL	2x10	1 Wechsler Memory Scale 1a Logical memory subtest 1b Associate learning subtest 2 Boston Naming test 3 Controlled Oral Word Association test 4 Trail making test 5 Finger Tapping test 6 Wechsler Adult Intelligence Scale-revised (composite of information, vocabulary and similarities sub-tests) 7 Benton Visual Retention test	Folic acid seemed to reduce performance on associate learning subtest of Wechsler Memory Scale (p=0·08) and Trails B (p=0·08).
Folic acid with other B vitamins							
Eussen et al[12]	24 weeks	162	82 (5)	Mini-Mental State Examination ≥ 19, ≥70 years, suboptimal vitamin B12 status (B12 100–200 pmol/L or B12 200–300 pmol/L, methylmalonic acid ≥0·32 µmol/L, creatinine ≤120 µmol/L)	0·4	Domains based on clustering of similar tests 1 Attention 2 Construction 3 Sensomotor speed 4 Memory 5 Executive function	Compared with placebo or to vitamin B_{12} only. No effect of folic acid on cognitive domains.
McMahon et al[13]	2 years	253	74 (6)	≥65 years, homocysteine ≥13 µmol/L	1	1 Mini-Mental State Examination 2 Wechsler Paragraph Recall 3 Category Word Fluency 4 Rey Auditory Verbal Learning 4a composite of trials 1-5 4b trial 7 5 Raven's Progressive Matrices 6 Controlled Oral Word Association 7 Reitan Trail Making, part B 8 Composite score of all tests	General trend towards reduced performance on tests. In crude analyses significance was reached for the Reitan Trail Making test part B) (7% slower, 95% CI 2 to 13%) and Wechsler Paragraph Recall test (mean difference –1·19, 95% CI –2·30 to –0·04). After adjustment for baseline performance, sex, and education, the composite score of all tests was lower in the folic acid group than in the placebo group (–0·11, 95% CI –0·22 to 0·00).
Stott et al[14]	1 year	167	75 (6)	Mini-Mental State Examination ≥ 19, ischaemic vascular disease, ≥65 years, red-blood-cell folate ≥280 ng/mL, vitamin B12 ≥250 pg/mL	2·5	1 Telephone Interview for Cognitive Status 2 Letter Digit Coding	No effect

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Bryan et al[15]	5 weeks	104	51 (20)	Healthy women	0·75	1 Boxes test 2 Digit Symbol Coding, 120 s 3 Symbol search 4 Digit Span Backward 5 Letter-Number Sequencing 6 Rey Auditory-Verbal Learning test 6a Immediate recall 6b Delayed recall 7 Digit Symbol Coding, symbol recall 8 Activity recall 9 Stroop test 10 Self-ordered pointing task 11 Uses for common objects 12 Trail Making test 13 Verbal Fluency test 14 Excluded Letter Fluency 15 Wechsler Adult Intelligence Scale - III Vocabulary 16 Spot-the-Word test	Folic acid reduced Verbal Fluency performance (p<0·05). When stratifying by age, folic acid improved Rey Auditory-Verbal Learning test (recognition task) in older participants (>65 years, p<0·05).
Toole et al[16]	2 years	3097	66 (10)	Previous stroke, homocysteine >~9 μmol/L	2·5 or 0·02	1 Mini-Mental State Examination	No effect
Vital Trial Collaborative Group[17]	12 weeks	128	Range 56·89	Mini-Mental State Examination score 12–26 or mild cognitive impairment assessed by modified Telephone Interview of Cognitive Status	2	1 Mini-Mental State Examination 2 Alzheimer's Disease Assessment Scale (cognitive part)	No effect
Lewerin et al[18]	4 months	171–179	76 (4)	Community-dwelling	0·8	1 Digit Span Forward 2 Digit Span Backward 3 Identical forms 4 Visual reproduction 5 Synonyms 6 Block design 7 Digit Symbol, 90s 8 Thurstone's Picture Memory test 9 Figure Classification	Folic acid improved performance on identical forms and synonyms (p<0·05).
Obeid et al[19]	45 days	69	80 (6)	Mini-Mental State Examination score >15, >65 years	5·0 orally or 1·1 (intravenously three times a week for 3 weeks)	1 Structured Interview for Diagnosis of Dementia of Alzheimer Type, Multi-infarct Dementia and Dementia 2 Orientation abilities 3 Memory 4 Intellectual abilities	No treatment effect reported, only differences in performance within groups

DSM-III-R=Diagnostic and Statistical manual of Mental Disorders, 3rd edition revised.

Table 1: **Summary of randomised controlled trials examining effect of folic-acid-containing supplements on cognitive function**

they had been allocated placebo. All staff, including all authors, were unaware of group assignment until completion of the trial and after data analyses.

We assessed cognitive function using five separate tests used in the Maastricht Aging Study.[21] These five tests were used to construct five a-priori-defined cognitive domains: memory, sensorimotor speed, complex speed, information processing speed, and word fluency.[22] Descriptions of the tests are in panel 1.[23–27] Although not part of the cognitive tests used for our study outcome, we used the Mini-Mental State Examination[9] to screen for participants with possible dementia, defined as a score of less than 24 points.

All participants underwent the measurements after an overnight fast, followed by a glass of fruit juice and a bread product for breakfast. Two trained research assistants oversaw the tests during a 40-min session; they used standard wording to instruct participants. A third research assistant periodically observed the testing to ensure that the two research assistants did not deviate from the protocol. All cognitive tests were done in the same room with the same props. We repeated cognitive testing at the end of the study using variations of the tests used at baseline (parallel versions). We repeated the verbal fluency test because the validity of a parallel version of the animal-naming part of the test has not been established.

Fasting venous blood was processed and samples were stored at −80°C. We measured serum folate, erythrocyte folate, serum vitamin B_{12}, plasma total homocysteine, plasma vitamin B_6, serum creatinine, and lipids as described elsewhere.[22] The C677T polymorphism in the gene encoding methylenetrahydrofolate reductase (*MTHFR*)[28] and apolipoprotein E genotype were determined by PCR of DNA and restriction digestion with *Hin*Fl and *Hha*1, respectively.

Self-reported medical history, including current drug use and smoking habits, were ascertained by questionnaire

and reviewed by a research assistant with the participant. Education was grouped according to highest attained level.[29] Height and weight were measured and body-mass index calculated. Blood pressure was measured with an automated meter (Dinamap Compact Pro 100, General Electric). Eight blood-pressure measurements were taken and the average calculated. We used a food-frequency questionnaire to estimate dietary folate intake during the past 3 months.

We measured genotype and attained educational level at the beginning of the study. Plasma total homocysteine, serum folate, and vitamin B_{12} concentrations and information about medical status and drug use were recorded yearly and all other measurements were taken at the beginning and end of the study.

Statistical analyses

Our sample size calculation was based on the mean intima-media thickness progression of the common carotid artery (the primary endpoint of the FACIT trial). We assumed that if the progression of the mean carotid intima-media were 0·01 mm (SD 0·06 mm), then 251 participants would be needed in each group to detect a difference of 0·015 mm (power 80%, two-sided α=0·05). We assumed that 30–40% of the population could be lost to follow-up.

The cognitive domains were constructed with *Z* scores (panel 2).[21] Sensorimotor speed measures basic speed, and shows direct stimulus-response connections with little central processing, whereas complex speed measures time needed for higher-order information processing. As other investigators have done,[30,31] we present global cognitive function (an average of the domains). The test scores at the beginning and end of the study were pooled to calculate the grand mean and SD per test; this information was used to calculate the *Z* scores (panel 2). At baseline, one participant missed 50% or more of the subtests for complex speed and three participants did not have an information processing speed score. These participants were assigned the median score of these domains of the total population at baseline. 17 participants lost to follow-up were assigned the median test score of the total population at the end of the study. Analyses were done on an intention-to-treat basis with SPSS 11.0. No adjustments were made for multiple testing.

The outcome of this study was the difference between the folic acid and placebo groups in the 3-year change in performance for memory, sensorimotor speed, complex speed, information-processing speed, and word fluency.

Panel 1: Description of cognitive function tests

Word learning test[23]
Measures the storage and retrieval of newly acquired verbal information. Participants were instructed to memorise 15 commonly used monosyllabic words. The words were printed on a card and were presented in a fixed sequence for 2 s. Immediately after the 15 words are presented, the participants are asked to recall the words. This procedure was done three times. 20 min after presentation of the words, participants were prompted to recall the 15 words. The maximum and total number of correctly repeated words of the immediate recall tests were recorded, as well as the number of correctly repeated words in the delayed recall test.

Concept shifting test[24]
A timed test with four subtests that measure the ease of switching between two psychological concepts. Each subtest was printed on one sheet of paper, which contained 16 circles (15 mm diameter) arranged in a larger circle (16 cm diameter). For the first subtest, participants were asked to cross off the circles in numerical and alphabetical order (eg, 1, A, 2, B, 3, C, etc).

Stroop colour-word test[25]
Measures selective attention and susceptibility to behavioural interference and consists of three subtests. Each subtest was presented on a separate sheet containing four rows of ten columns of colour names of coloured blocks. For the first subtest, participants were instructed to read words printed in black ink (words were "red", "blue", "green", and "yellow"). Participants were asked to name coloured blocks in the second subtest, for the final subtest, participants were asked to name the colour of the ink, rather than read the word (eg, say blue when the word "RED" was printed in blue ink).

Verbal fluency test[26]
Measures word fluency or the ability to draw on one's encyclopaedic memory in a strategic manner. Participants were asked to name as many animals as possible in 1 min. This test indicated the amount of organisation among clusters of related words (eg, pets, zoo animals, etc).

Letter digit substitution test[27]
Assesses general speed of visual information processing. Nine different letters were assigned a unique number (1–9) in a key at the top of the form. The participants were presented with a random series of letters in cells and were instructed to add the corresponding digit to the letters. The number of correctly copied corresponding digits in 90 s was recorded.

We used the *t* test to determine whether the change in cognitive performance differed between treatment groups. We did all analyses without knowledge of follow-up folate or homocysteine concentrations. The treatment code was broken once an independent statistician had verified the data and all authors had formally approved the tables showing the main effects.

In secondary analyses, we determined whether the effect of folic acid supplementation was dependent on initial concentrations of folate or homocysteine or *MTHFR* C677T genotype. We determined the effect of folic acid

Panel 2: Construction of cognitive domains with *Z*-scores

$$\text{Memory}=(Z_{\text{15-Word Learning test 'total immediate recall'}}+Z_{\text{15-Word Learning Test 'maximum immediate recall'}}+Z_{\text{15-Word Learning Test 'delayed recall'}})/3$$

$$\text{Sensorimotor speed}=-(Z_{\text{Concept Shifting test 'empty'}}+Z_{\text{Concept Shifting test 'numbers'}}+Z_{\text{Concept Shifting test 'letters'}}+Z_{\text{Stroop Color-Word test 'word reading'}})/4$$

$$\text{Complex speed}=-(Z_{\text{Concept Shifting test 'numbers and letters'}}+Z_{\text{Stroop Color-Word test 'naming ink color'}})/2$$

$$\text{Information processing speed}=Z_{\text{Letter Digit Substitution test}}$$

$$\text{Word fluency}=Z_{\text{Verbal Fluency test}}$$

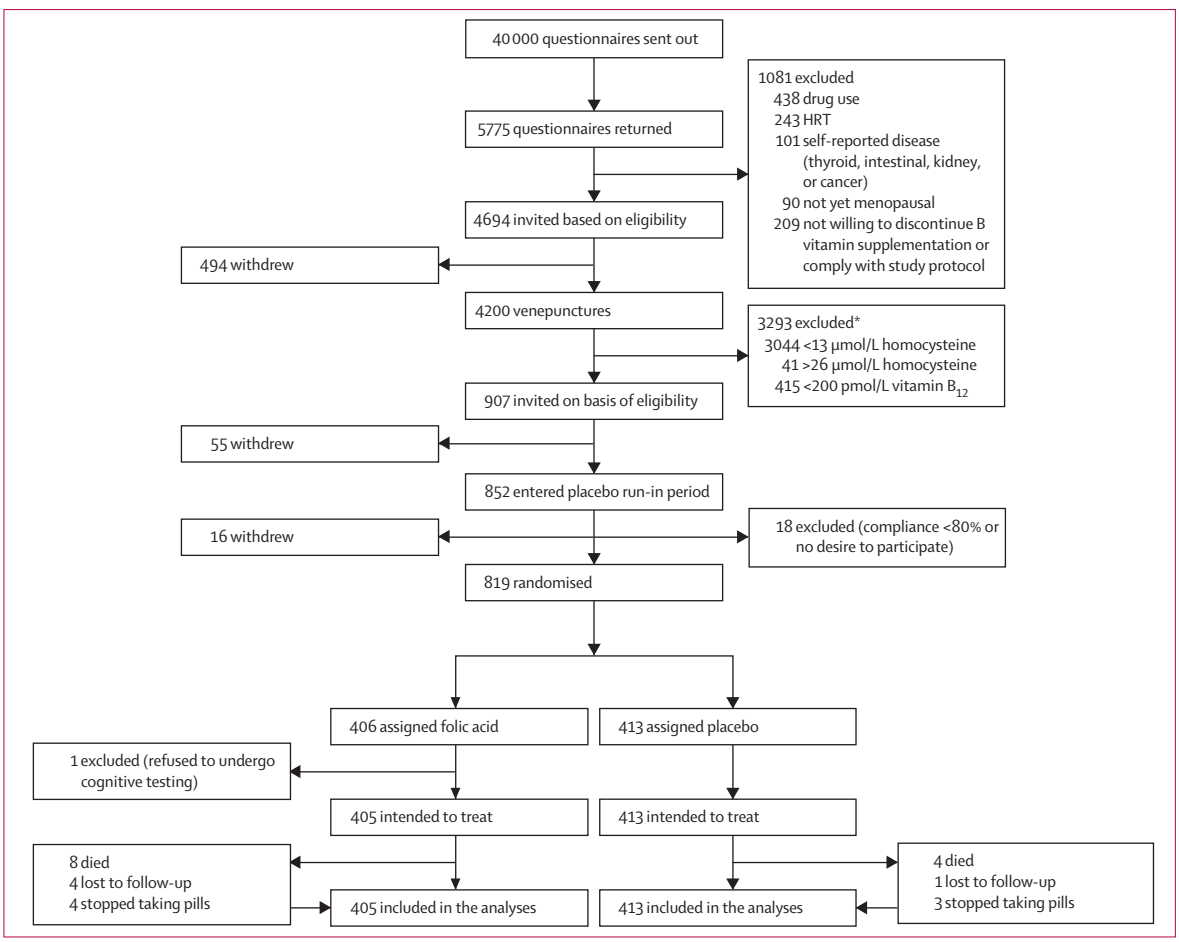

Figure: **Trial profile**
HRT=hormone replacement therapy. *Participants might have been excluded for multiple reasons.

supplementation per stratum (median cut-off) using an independent sample *t* test. We used linear regression models to examine whether the difference in treatment effects between strata was significant.

Role of the funding source

The sponsors had no role in the design or implementation of the study, data collection, data management, data analysis, data interpretation, or in the preparation, review, or approval of the manuscript. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Patients were randomised between November, 1999, and April, 2001, and the study was undertaken from September, 2000, to December, 2004. The figure shows the trial profile. Five participants allocated folic acid treatment reported adverse events: forgetfulness, sun allergies, weight gain, tinnitus, and dark urine. Adverse effects reported in the placebo group (n=7) were muscle aches, headaches, weight gain, queasiness, bitter taste in mouth, and skin irritations. The participant with headache complaints dropped out of the trial prematurely. 17 participants (2%) did not return for the cognitive function measurements after 3 years, and six participants stopped treatment prematurely. The proportion of participants lost to follow-up or who stopped treatment early did not differ between the groups (p=0·25). Apart from these participants, the compliance of the participants was high, with 99% of the capsules reportedly consumed. Table 2 shows baseline clinical characteristics of the participants.

In both groups, plasma total homocysteine concentrations at baseline were lower than at screening, a likely result of regression to the mean (table 3). After 3 years, serum folate concentrations were significantly higher in the folic acid group than in the placebo group (table 3).

Baseline scores on the cognitive tests and domains were similar between the two groups (table 4). At baseline, one participant in the folic acid group and six in the placebo group scored less than 24 points on the Mini-Mental State Examination (p=0·85). Whereas sensorimotor speed, information-processing speed, and complex speed declined significantly during the 3-year study in the placebo group,

the rate of decline was slower in these domains in the folic acid group (table 5). Both groups improved on memory tests, because of procedural learning effects. However, the improvement was significantly greater in the folic acid group than in the placebo group.

The 3-year change in cognitive function was significantly better in the folic acid group than in the placebo group in terms of information-processing speed. Folic acid did not affect sensorimotor speed, complex speed, or word fluency. Global cognitive function, defined as the average of the five domains, improved significantly (table 5). Performance on the Mini-Mental State Examination was not affected by folic acid group (p=0·63). The median score for both groups after 3 years was 29 points (IQR 28–30) ranging from 21 to 30 points in the folic acid and 16 to 30 points in the placebo group.

In addition to memory and information-processing speed, sensorimotor speed improved significantly (p<0·05) when other imputation techniques were used (eg, last value carried forward, expectation maximisation), when 17 participants lost to follow-up were excluded from the analyses, and when seven participants with initial Mini-Mental State Examination scores of less than 24 points were excluded from the analyses. Finally, at baseline, a greater proportion of participants with a low educational level, an important determinant of cognitive performance, were randomised into the folic acid group. Additionally, a higher proportion of participants in the folic acid group had dyslipidaemia and self-reported vascular disease. Our results did not change when we adjusted for these variables.

51 participants received the same treatment and lived in the same households. Hence these observations were not independent of one another. When partners were excluded from the analyses, the results did not change, except that folic acid supplementation significantly improved sensorimotor speed (difference in *Z* scores 0·079 [95% CI 0·014–0·145]).

To show the relevance of our findings we compared the regression coefficient of age—adjusted for sex, education, and treatment calculated with linear regression models with initial performance as the dependent variable—with the treatment effect. 3-year folic acid supplementation confers an individual the performance of someone 4·7 years younger for memory (95% CI 1·1–8·3), 1·7 years younger for sensorimotor speed (–0·04 to 3·4), 2·1 years younger for information processing speed (0·4–3·7), and 1·5 years younger for global cognitive function (0·1–2·8). Of our test battery, memory—specifically delayed memory—is the most clinically relevant test. We showed that 3-year folic acid supplementation improved performance on the delayed recall sub-test of the 15 word learning test by 0·47 words (95% CI 0·14–0·79, p=0·005). This improvement is similar to a performance of an individual 6·9 years younger (95% CI 2·1–11·8).

The effect of folic acid supplementation was not modified by initial folate status or *MTHFR* C677T genotype.

	Folic acid (n=405)	Placebo (n=413)
Age (years)	60 (5)	60 (6)
Male	294 (72%)	292 (70%)
High / middle / low education	154 (38%)/144 (36%)/107 (26%)	169 (41%)/168 (41%)/76 (18%)
Mini-mental state examination (points)	29 (28–30)	29 (28– 30)
Range (points)	18–30	15–30
MTHFR 677T allele 0 , 1 , 2*	143 (36%), 187 (46%), 73 (18%)	168 (41%), 191 (46%), 52 (13%)
Vitamin B_{12} (pmol/L)	290 (239–366)	286 (247–363)
Vitamin B_6 (nmol/L)†	37·8 (28·2–49·8)	368·4 (28·2–48·3)
Creatinine (mmol/L)	92·7 (12·5)	92·4 (12·0)
Total cholesterol (mmol/L)	5·8 (1·1)	5·8 (1·1)
LDL cholesterol (mmol/L)†	4·0 (1·0)	4·0 (1·0)
HDL cholesterol (mmol/L)	1·2 (0·3)	1·2 (0·4)
Dyslipidaemia‡	156 (39%)	138 (33%)
ApoE ε 4 allele, 0 , 1 , 2§	271 (67%), 122 (30%), 11 (3%)	282 (69%), 116 (28%), 12 (3%)
Systolic blood pressure (mm Hg)*	133 (16)	133 (16)
Diastolic blood pressure (mm Hg)*	77 (8)	77 (9)
Hypertension*¶	94 (23%)	88 (21%)
Body-mass index‖	26·6 (3·6)	26·5 (3·6)
Current smokers	84 (21%)	83 (20%)
Diabetes mellitus	12 (3%)	14 (3%)
Self-reported cardiovascular disease**	58 (14%)	39 (9%)

MTHFR=methylenetetrahyrdofolate reductase. *Data available for 403 participants in folic acid group and 411 participants in placebo group. †Data available for 411 participants in placebo group. ‡Total cholesterol >6·5 mmol/L, HDL cholesterol <0·9 mmol/L or use of lipid-lowering drugs. §Data available for 403 participants in folic acid group and 410 participants in placebo group. ¶Systolic blood pressure ≥160 mm Hg, diastolic blood pressure ≥95 mm Hg or use of antihypertensive drugs. ‖body-mass index=weight in kg divided by the square of the height in metres. **Diagnosis of angina pectoris, myocardial infarction, arrhythmia, stroke, or peripheral arterial disease, or having undergone angioplasty, coronary bypass surgery, or aortic aneurysm surgery. Data are mean (SD), median (IQR) or number (%) unless otherwise indicated.

Table 2: **Baseline clinical characteristics**

Compared with placebo, participants with initial plasma total homocysteine concentrations greater than the population median of 12·9 μmol/L showed a greater improvement in information processing speed than participants with concentrations lower than the population median (interaction term p=0·034). Information-processing speed of the latter group improved by 0·013, (95% CI -0·086 to 0·111), whereas participants with higher homocysteine concentrations improved by 0·166, (0·064–0·267). Outcomes of the other four domains and global cognitive function were not affected by initial plasma total homocysteine concentrations.

In post-hoc analyses, we examined whether low concentrations of vitamin B12 modified the effect of folic acid supplementation on cognitive performance. Folic acid supplementation improved sensorimotor speed (difference in *Z* score 0·112, 95% CI 0·001–0·223) and information processing speed (0·190, 0·055–0·325) in 230 participants with initial low or normal concentrations of vitamin B_{12} (<250 pmol/L), but not in 588 participants with vitamin B_{12} concentrations of 250 pmol/L or greater (0·046, –0·033 to 0·126 and 0·048, –0·036 to 0·131, respectively).

	Folic acid	Placebo	p
Serum folate (nmol/L)*			
Baseline	12 (9–15)	12 (10–15)	..
Year 1	53 (44–86)	12 (9–15)	<0·001
Year 2	50 (42–85)	12 (9–15)	<0·001
Year 3	76 (50–103)	13 (10–17)	<0·001
Erythrocyte folate (nmol/L)†			
Baseline	630 (494–829)	671 (535–815)	..
Year 3	2043 (1741–2469)	697 (546·1–888)	<0·001
Dietary folate intake (µg per day)‡			
Baseline	192 (159–238)	195 (158–242)	..
Year 3	182 (152–218)	179 (152–224)	0·946
Plasma total homocysteine (µmol/L)§			
Baseline	13·0 (11·6–14·7)	12·9 (11·4–14·8)	..
Year 1	9·4 (8·3–10·5)	12·4 (10·7–14·6)	<0·001
Year 2	9·6 (8·4–10·9)	12·5 (11·0–15·0)	<0·001
Year 3	10·1 (9·0–11·3)	13·4 (11·5–15·2)	<0·001

Data are median (IQR). At the time of our study folic acid fortification of foods was prohibited in the Netherlands. p values based on non-parametric tests. *At baseline, data available for 405 participants in folic acid group and 413 participants in placebo group. At Year 1, data available for 397 participants in folic acid group and 407 participants in placebo group. At Year 2, data available for 389 participants in folic acid group and 403 participants in placebo group. At Year 3, data available for 394 participants in folic acid group and 406 participants in placebo group. †At baseline, data available for 405 participants in folic acid group 413 participants in placebo group. At Year 3, data available for 394 participants in folic acid group and 406 participants in placebo group. ‡At baseline, data available for 405 participants in folic acid group and 413 participants in placebo group. At Year 3, data available for 394 participants in folic acid group and 408 participants in placebo group. §At baseline, data available for 405 participants in folic acid group and 412 participants in placebo group. At Year 1, data available for 397 participants in folic acid group and 404 participants in placebo group. At Year 2, data available for 390 participants in folic acid group and 404 participants in placebo group. At Year 3, data available for 393 participants in folic acid group and 406 participants in placebo group.

Table 3: **Folate status and total homocysteine concentrations throughout the study**

	Folic acid (n=405)	Placebo (n=413)
Word learning test		
Total of three immediate recall trials (number of words)	26·9 (5·9)	26·8 (5·4)
Maximum of three immediate recall trials (number of words)	11·2 (2·2)	11·2 (2·0)
Delayed recall (number of words)	8·9 (2·8)	9·0 (2·7)
Concept shifting test		
Empty (s)*	5·37 (1·16)	5·43 (1·28)
Numbers (s)	23·26 (5·82)	23·40 (6·07)
Letters (s)†	27·01 (7·40)	27·46 (9·51)
Numbers and letters (s)‡	35·95 (12·28)	36·34 (13·35)
Stroop colour-word test		
Word reading (s)*	16·34 (2·75)	16·38 (3·09)
Naming colour of ink (s)§	42·62 (10·65)	43·68 (11·79)
Letter digit substitution test, number of digits in 90 s¶	48·86 (8·96)	48·24 (8·99)
Verbal fluency test, number of words	24·8 (6·3)	24·1(5·7)

Performance did not differ between two groups. *Data available for 412 participants in placebo group. †Data available for 403 participants in folic acid group and 411 participants in placebo group. ‡Data available for 409 participants in placebo group. §Data available for 404 participants in folic acid group and 411 participants in placebo group. ¶Data available for 410 participants in placebo group. Data are mean (SD).

Table 4: **Performance on cognitive function tests at baseline**

Discussion

In 818 older adults, daily oral folic acid supplementation for 3 years beneficially affected global cognitive function, and specifically memory, and information processing: functions that are sensitive to ageing.[1] The decline in memory seen with ageing is generally preceded, and might be affected, by a decline in speed functions.[32] Nonetheless, folic acid supplementation might beneficially affect both memory and speed simultaneously, since high concentrations of homocysteine have been associated with atrophy of the hippocampus, an area of the brain which is important for memory consolidation.[33] Complex speed, a domain sensitive to ageing,[1] was not affected by folic acid supplementation. The effect of folic acid might be restricted to basic aspects of speed and information processing, rather than high order information processing. Word fluency was not affected by folic acid supplementation, perhaps not surprisingly, because encyclopaedic memory is a component of crystallised intelligence that stays relatively intact as one grows older.[34]

Our study might have yielded demonstrable effects of folic acid on cognitive function because we used sensitive tests that exist in parallel versions. We also improved the robustness of the underlying cognitive constructs by clustering raw test scores for several tests in compound performance measures. This procedure decreased variation associated with the individual tests. Finally, clustering of raw tests scores limited our cognitive performance outcomes to five a-priori defined outcomes.

By contrast with other trials, we were able to detect an effect of folic acid on several cognitive functions, for several reasons. First, assuming that high plasma total homocysteine concentrations are a causal risk factor for cognitive decline, we selected a population likely to benefit from folic acid supplementation. Second, we had a fairly large study population and supplemented for quite a long period. Third, although we did not attempt to measure the prevalence of dementia at baseline nor its incidence during the duration of the trial, our population is unlikely to have included many cognitively impaired or demented participants, since the general performance on a dementia screening test such as the Mini-Mental State Examination was high, both at the beginning and end of the study. That treatment with folic acid or other B vitamins might feasibly be too late in populations with mild cognitive impairment and dementia. Finally, sensitive tests such as our own—not the commonly used Mini-Mental State Examination, which is a dementia screening device—were needed to detect the subtle effects of B vitamins on cognitive ageing. Importantly, given the general scarcity of positive findings from other trials (table 1) and the multiple comparisons made in our trial, our results need to be confirmed by other investigators to ascertain whether the significant positive effects of folic acid on cognitive performance were due to type 1 error.

A strength of our study is the low attrition rate; a high attrition rate might have biased our findings of cognitive change, since participants with poor cognitive function are likely to withdraw from studies.[35] In our study, the 12 participants in the folic acid group and five participants in the placebo group who did not return for the end measurements had low scores only on baseline tests of

	Folic acid(n=405)				Placebo (n=413)				Folic acid vs placebo	
	Year 0	Year 3	3-year change in cognitive performance,	p*	Year 0	Year 3	3-year change in cognitive performance, mean (SD)	p*	Cognitive change attributed to folic acid, mean difference (95% CI)	p†
Global cognitive function, mean (SD) *Z* score	0·006 (0·673)	0·073 (0·694)	0·067 (0·338)	<0·0001	–0·048 (0·672)	–0·031 (0·701)	0·017 (0·332)	0·287	0·050 (0·004 to 0·096)	0·033
Memory, mean (SD) *Z* score	–0·207 (0·959)	0·273 (0·965)	0·480 (0·724)	<0·0001	–0·206 (0·883)	0·142 (0·961)	0·348 (0·737)	<0·0001	0·132 (0·032 to 0·233)	0·010
Sensorimotor speed, mean (SD) *Z* score	0·054 (0·706)	0·011 (0·753)	–0·042 (0·458)	0·063	0·019 (0·836)	–0·087 (0·819)	–0·106 (0·490)	<0·0001	0·064 (–0·001 to 0·129)	0·055
Complex speed, mean (SD) *Z* score	0·053 (0·803)	0·026 (0·868)	–0·027 (0·651)	0·405	–0·008 (0·879)	–0·072 (0·865)	–0·064 (0·593)	0·029	0·037 (–0·049 to 0·122)	0·40
Information processing speed, *Z* score	0·093 (1·008)	0·021 (0·967)	–0·072 (0·513)	0·005	0·024 (1·008)	–0·135 (1·008)	–0·159 (0·517)	<0·0001	0·087 (0·016 to 0·158)	0·016
Word fluency, mean (SD) *Z* score	0·038 (1·056)	0·036 (1·029)	–0·002 (0·864)	0·961	–0·070 (0·959)	–0·002 (0·953)	0·068 (0·859)	0·108	–0·070 (–0·188 to 0·048)	0·245

* One sample *t* test(0). †Independent sample *t* test.

Table 5: **Change in cognitive performance within groups during 3 years and difference in cognitive change attributed to folic acid supplementation**

memory (0·558 *Z* score, 95% CI 0·116–1·000). Their absence from year-3 tests is unlikely to have affected our estimates for several reasons: the effect of folic acid supplementation on memory was not dependent on baseline performance on the memory tests (data not shown), the number of participants lost to follow-up was minimal, and the effect estimates based on participants with follow-up data were similar to the intention-to-treat analyses. A second strength was the standardised test conditions that reduced variation due to factors such as caffeine and varying breakfasts.[36]

Our study also had some limitations. First, we studied participants with high plasma total homocysteine concentrations: 3044 out of 4200 participants were excluded from the study because of low plasma total homocysteine concentrations. Thus, the effect of folic acid supplementation on cognitive function might be greater than would be expected in populations with lower plasma total homocysteine concentrations—eg, in countries such as the USA, with mandated fortification of flour with folic acid. Second, our findings pertain only to vitamin B12-replete individuals. Suggestions have been made that folic acid supplementation exacerbates neurological symptoms in people with vitamin B_{12} deficiency.[37] The possibility of folic-acid-mediated exacerbation of neuropathological disorders in people with low concentrations of vitamin B_{12} needs to be addressed by studies that monitor both vitamin B_{12} status and neurological function. As an improvement to our own study, transcobalamin in addition to vitamin B_{12}, should be measured, because transcobalamin is a better marker of vitamin B_{12} status than is vitamin B_{12} itself.

Will folic acid supplementation lead to a reduced incidence of dementia? Whereas some have argued that cognitive decline is the beginning of a continuum leading to dementia,[38] others have argued that the cause of age-related cognitive decline differs from that of dementia[39] and that age-related cognitive decline is not an early state of mild cognitive impairment or dementia.[2] Cognitive tests differ in their ability to identify individuals who worsen to more advanced states such as mild cognitive impairment or dementia. Of our test battery, memory is the most clinically relevant domain. Memory can be used to distinguish between cognitively normal and cognitively impaired people.[2] Memory storage (delayed recall), in particular, can distinguish between people with non-progressive mild cognitive impairment and preclinical Alzheimer's disease.[40] Although folic acid improved performance on tests of memory, including delayed recall, additional research is needed to determine whether folic acid supplementation can reduce the risk of mild cognitive impairment or Alzheimer's disease.

We have shown that 3-year folic acid supplementation improves performance on tests that measure information-processing speed and memory, domains that are known to decline with age, in older adults with raised total homocysteine concentrations. Randomised, controlled trials are underway to examine the effect of homocysteine-lowering on recurrent vascular disease and cognitive function assessed by the Mini-Mental State Examination or modifications thereof; these and other homocysteine-lowering trials should include sensitive measures of cognitive function. Additionally, trials similar to our own should be repeated in other populations to provide greater insight into the clinical relevance of folic acid supplementation, such as in populations with mild cognitive impairment and dementia.

Contributors

All authors participated in the study design, study implementation, and in the interpretation of the results.

Conflict of interest statement

Jane Durga currently works at Nestle Research Center in Lausanne, Switzerland and Petra Verhoef currently works at the Unilever Food and Health Research Institute in Vlaardingen, the Netherlands. The work at both food companies entails examining the health benefits of a variety of

food ingredients, including folic acid. However, the study reported in the current manuscript was completed and submitted to *The Lancet* before the authors joined the companies, when they were still employed by Wageningen University and Wageningen Centre for Food Sciences. All authors declare that they have no conflict of interest.

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