Oral folic acid and vitamin B-12 supplementation to prevent cognitive decline in community-dwelling older adults with depressive symptoms—the Beyond Ageing Project: a randomized controlled trial

Janine G Walker, Philip J Batterham, Andrew J Mackinnon, Anthony F Jorm, Ian Hickie, Michael Fenech, Marjan Klijakovic, Dimity Crisp, and Helen Christensen

ABSTRACT

Background: Evidence remains unclear as to whether folic acid (FA) and vitamin B-12 supplementation is effective in reducing depressive symptoms.

Objectives: The objective was to determine whether oral FA + vitamin B-12 supplementation prevented cognitive decline in a cohort of community-dwelling older adults with elevated psychological distress.

Design: A randomized controlled trial (RCT) with a completely crossed 2 × 2 factorial design comprising daily oral 400 µg FA + 100 µg vitamin B-12 supplementation (compared with placebo), physical activity promotion, and depression literacy with comparator control interventions for reducing depressive symptoms was conducted in 900 adults aged 60–74 y with elevated psychological distress (Kessler Distress 10–Scale; scores >15). The 2-y intervention was delivered in 10 modules via mail with concurrent telephone tracking calls. Main outcome measures examined change in cognitive functioning at 12 and 24 mo by using the Telephone Interview for Cognitive Status–Modified (TICS-M) and the Brief Test of Adult Cognition by Telephone (processing speed); the Informant Questionnaire on Cognitive Decline in the Elderly was administered at 24 mo.

Results: FA + vitamin B-12 improved the TICS-M total (P = 0.032; effect size d = 0.17), TICS-M immediate (P = 0.046; d = 0.15), and TICS-M delayed recall (P = 0.013; effect size d = 0.18) scores at 24 mo in comparison with placebo. No significant changes were evidenced in orientation, attention, semantic memory, processing speed, or informant reports.

Conclusion: Long-term supplementation of daily oral 400 µg FA + 100 µg vitamin B-12 promotes improvement in cognitive functioning after 24 mo, particularly in immediate and delayed memory performance. This trial was registered at clinicaltrials.gov as NCT00214682.

INTRODUCTION

Cognitive impairment is common in later life, with prevalence rates ranging from 2% to 29% (1, 2). It is associated with impaired neuropsychiatric, physical and social functioning, and reduced quality of life (3, 4) and predicts conversion to dementia (5). Effective prevention strategies are needed to protect individuals from decline or at least to minimize any adverse effects of cognitive impairment (6). A first step in the development of such programs is to identify interventions that prevent cognitive impairment in community-dwelling older adults (7). To date, the development and evaluation of such programs has been minimal.

One candidate intervention is oral FA combined with vitamin B-12 supplementation. Two theories explain how such an intervention may prevent cognitive impairment and dementia. The first is by lowering homocysteine concentrations (8–10), whereas the second purports that supplementary FA and vitamin B-12 may operate by reducing vascular and other metabolic risk factors (9). To date, the strength of the evidence drawn from RCTs is equivocal with regard to the efficacy of FA and vitamin B-12 as a treatment of cognitive impairment or dementia (10–17). Systematic reviews of the literature indicate that trials involving cognitively impaired individuals who received a combination of vitamin B complex + FA supplements or FA alone showed improvements in memory, attention efficiency, motor speed, and visual conceptual and vasomotor tracking compared with control individuals (10, 18). However, less is known as to whether FA and vitamin B-12 supplementation is effective for...
the prevention of negative cognitive changes, particularly in community-dwelling older adults (10, 19).

The present article reports a secondary analysis of the FA and vitamin B-12 (FA + vitamin B-12) supplementation intervention arising from a large-scale RCT designed primarily to investigate physical activity, mental health literacy, and FA + vitamin B-12 supplementation as preventive interventions for depression in an older population with elevated depressive symptoms (20). Those with elevated depressive symptoms are an important cohort to target because of evidence that late-life depression is associated with increased risk of cognitive impairment (21), with severe compared with mild depressive symptoms representing a greater risk of mild cognitive impairment (22).

We hypothesized that oral FA (400 µg/d) + vitamin B-12 (100 µg/d) supplementation would slow the rate of negative changes in cognitive function in a sample of community-dwelling individuals with elevated depression symptoms compared with placebo. Given the relative youth of our sample and the short follow-up time (24 mo), we expected only small negative cognitive changes to be observed. Indeed, it was possible that short-term increases in performance levels due to practice effects would be observed, because these are common in longitudinal studies in community-dwelling older adults (23). We predicted the extent of any negative changes (either a deterioration in performance levels, or a reduced improvement in performance due to practice effects) would be greater for individuals in the placebo condition than in those taking FA + vitamin B-12 supplementation.

SUBJECTS AND METHODS

Study design

The present study was an RCT that investigated potentially effective interventions for reducing depressive symptoms in older adults with a 2 × 2 × 2 factorial design [(FA + vitamin B-12 supplementation compared with placebo tablet) × (physical activity promotion compared with comparator control, ie, information regarding nutrition for older adults) × (mental health literacy compared with comparator control, ie, pain and arthritis management information)]. Participants were randomly assigned to 1 of the 8 intervention programs arising from the combination of active or comparison conditions. The design and randomization process are reported elsewhere (20). Most outcomes were assessed at 5 time points: baseline, 6 wk, and 6, 12, and 24 mo. The cognitive functioning variables were assessed at baseline and at 12 and 24 mo.

Recruitment occurred between 22 October 2005 and 4 September 2006, with the 24-mo intervention and data collection occurring from 4 January 2006 to 18 September 2008. The Human Research Ethics committees at the Australian National University, Australian Capital Territory Health Department, and The University of Sydney, Australia, approved the study. All participants provided written informed consent.

Participants

The population-based sample was recruited by a direct mailing of a screening survey and consent form to 105,000 randomly selected adults aged from 60 to 74 y whose names, addresses, and dates of birth were obtained from the mail lists provided by the Australian Electoral Commission; the sample comprised federal electorates in 2 cities, Canberra (Australian Capital Territory) and Sydney, and a rural location, Wagga Wagga (New South Wales). It was anticipated that initially recruiting in this manner would yield an exhaustive and representative sample because electoral registration is mandatory in Australia. The screening survey addressed a number of factors relevant to the study including demographic and health information, physical activity participation, vitamin B and FA intake, and psychological distress and was scored by research assistants. Individuals who met these criteria were then asked to provide a blood sample in a community-based blood collection site (Sonic Health Care Ltd) for additional health data and to determine that red blood cell folate and vitamin B-12 concentrations were in a healthy range for ethical reasons. Selected participants had elevated psychological distress as assessed by the K10 (scores ≥16) (24); did not engage in physical activity at public health–recommended levels as indicated by International Physical Activity Questionnaire scores; did not take FA, vitamin B-12, or vitamin B complex supplements; had no history of dementia, bipolar disorder, or current suicide risk; had competent literacy skills; and did not have a medical condition that would contraindicate exercise or FA use. Individuals with high likelihood of a depressive disorder with K10 scores of ≥30 were excluded (25). Those individuals with low concentrations of red blood cell folate (<250 nmol/L) and vitamin B-12 (<130 nmol/L) and abnormal thyroid stimulating hormone concentrations (0.35–5.0 mIU/L) were excluded because participation may have led to potentially adverse outcomes (20).

Interventions

Eligible participants were enrolled into an intervention program that was delivered over 24 mo in 10 modules. All interventions involved 5 brief telephone tracking calls over the first 5 wk and 5 more telephone calls at 4, 8, 13, 18, and 22 mo to ensure that the material and related tasks had been understood. With the exception of the FA + vitamin B-12 and placebo tablets, the first 5 modules of the interventions were delivered by mail during Weeks 1–5, followed by the remaining modules which were sent out at 4, 8, 13, 18, and 22 mo.

Dietary supplementation arm

FA + vitamin B-12 tablets were formulated as a daily oral dose of one tablet consisting of 400 µg FA and 100 µg vitamin B-12 (ABN57052101176; Matchland Pty Ltd) for the entire 24-mo period. After a safety review subsequent to a published RCT on the association between folate and colorectal adenomas (26), the protocol changed to 2 daily oral doses (200 µg FA + 50 µg vitamin B-12 each) from July 2007 (20). Adherence was monitored by telephone assessment at 14 time points and by blood assay at baseline and at 12- and 24-mo assessments.

Placebo tablets were manufactured by the same producers of the FA + vitamin B-12 tablets and were identical except for the omission of the active substances under investigation.

Demographic, physical, and mental health measures

Age, sex, years of education, and marital and employment status were established. A checklist identified vascular disease
and other health problems (27). The K10 was used to screen depression (28).

**Study outcome measures**

Cognitive function as well as concentrations of folate, vitamin B-12, and homocysteine were measured at baseline and at the 12- and 24-mo assessments.

Cognitive function was measured by using the TICS-M (29), which has a maximum total score of 39 and comprises 4 domains: 1) orientation; 2) registration, recent memory, and delayed recall; 3) attention/calculations; and 4) semantic memory, comprehension, and repetition. The TICS-M has a high proportion of the total score devoted to immediate and delayed recall, which affords it excellent discrimination in cognitive performance in the general population (29), and in the context of screening for dementia and mild cognitive impairment (30). Processing speed was measured by using the BTACT survey, which is a brief battery of key domains of cognitive function using tests that are sensitive to performance in community-dwelling adults ranging from young to middle-aged and older (31). Processing speed was measured with a backward-counting task in which the participant has 30 s to count backward quickly from 100 by ones. The total score represents the amount of numbers correctly reported in sequence, not counting errors (32). The reliability and validity of the BTACT has been shown to be good in community-dwelling samples, including in the elderly, with telephone administration yielding similar findings to usual in-person administration of standardized cognitive tests (32, 33).

The IQCODE was administered at 24 mo (34). It is a 26-item survey designed to measure cognitive decline and dementia in older adults. The questionnaire was completed by a relative or friend who had known the person for ≥2 y. The 5-point rating scale was designed to accommodate cognitive improvement as well as decline, with 1 indicating “much improved” to 5 indicating “much worse.” Ratings were averaged to give a 1–5 score, with 3 representing no change (35). The IQCODE has excellent reliability and validity and had been used effectively in clinical settings and epidemiologic research and in conventional cognitive screening tests as a screening tool (35).

Serum vitamin B-12, red blood cell folate, and homocysteine were measured at baseline and at the 12- and 24-mo assessments. A fluorescence polarization immunoassay was used for the quantitative determination of total t-homocysteine in plasma (AxSYM; Abbott Laboratories). Red blood cell folate and serum vitamin B-12 were measured by using chemiluminescent microparticle assays (Architect i2000; Abbott Laboratories). These allowed a check on whether participants were taking the supplements and to establish a change in homocysteine concentration that was consistent with other studies showing a protective effect.

Depression was assessed at baseline, 6 wk (within 1 wk), and at 6, 12, and 24 mo (all within 2 wk). Depressive symptoms were measured by using the Patient Health Questionnaire—9 (36).

**Randomization, sample size, power, and dropout**

Randomization followed the screening assessment, with the block size fixed at 8 and with strata comprising location, sex, and high (scores ≥19) and low (scores of 16–18) K10 (24) depression scores, by using an automated computerized system (AJM). Stratification was used as part of the randomization process to ensure an even distribution of these variables across the intervention groups. Furthermore, the Internet site random.org was used (conducted by JGW) to randomly allocate a label “A” or “B” to the FA + vitamin B-12 and placebo tablet bottles to ensure concealment of their content. Participants, interviewers, investigators, and the survey administrators were masked to active intervention and folie placebo allocation.

The flow of participants through the study is shown in Figure 1. From a total of 105,000 screening surveys delivered, 24,352 surveys (ie, 23.19% of surveys were returned, whereas 80,648 individuals did not respond to the initial mail contact) were received and screened. Of the screening surveys received, 14,684 (62.64%) individuals failed to meet the criteria for inclusion. The primary reason for exclusion from the intervention was self-reported low levels of distress (72.30% had K10 scores >16). Of those, 909 (3.73%) participants wanted to participate in the intervention component of the study, the study criteria, gave informed written consent, and were randomly assigned to 1 of 8 intervention combinations, with approximately half receiving FA + vitamin B-12 supplementation and the other half receiving the placebo. Calculations indicated that the sample size had 91% power to detect differences in treatment outcomes of 0.20 for the comparison of FA + vitamin B-12 with placebo, with an 𝛼 of 0.05 (20).

**Statistical analyses**

Mixed-model repeated-measures ANOVA was used to evaluate hypotheses concerning differential change between FA + vitamin B-12 and the placebo. Multiple comparisons were not adjusted for when examining the TICS-M subscales and total score because hypotheses were determined a priori (37). Within-person variation was modeled by using an unstructured covariance matrix. df were estimated by using Satterthwaite’s approximation (38). Models were developed for each of the cognitive outcome variables. The critical test of the effectiveness was the presence of an effect of the FA + vitamin B-12 supplementation relative to placebo over time—ie, showing that FA + vitamin B-12 supplementation improved cognitive functioning over time. Mixed models yield an intention-to-treat analysis by using all available measurement points for each participant under the assumption that withdrawal data are missing at random. Windows SPSS, version 15 (IBM Corporation), was used for all statistical analyses.

**RESULTS**

**Demographic characteristics and dropout**

Demographic differences at baseline are shown in Table 1. Those who received FA + vitamin B-12 had higher concentrations of serum vitamin B-12 than did those in the placebo group (Fr(1,908) = 5.33, P = 0.021). Of those who were recruited into the trial, the dropout rate was low, with only 123 (13.5%) participants withdrawing from the time of randomization to the 24-mo assessment. A total of 797 (87.7%) completed the 12-mo interview, and 752 (82.7%) completed the 24-mo interview. There were no significant differences in the proportions of...
FOLATE SUPPLEMENTATION AND COGNITIVE FUNCTIONING

<table>
<thead>
<tr>
<th>Group</th>
<th>Screened (n = 131)</th>
<th>Excluded (n = 3,847)</th>
<th>Randomly assigned (n = 909)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA MHL</td>
<td>Allocated to INT (n = 117)</td>
<td>Received INT (n = 114)</td>
<td>No INT (n = 1)</td>
</tr>
<tr>
<td>PA Pain</td>
<td>Allocated to INT (n = 114)</td>
<td>Received INT (n = 111)</td>
<td>No INT (n = 1)</td>
</tr>
<tr>
<td>PA Net MHL</td>
<td>Allocated to INT (n = 112)</td>
<td>Received INT (n = 111)</td>
<td>No INT (n = 0)</td>
</tr>
<tr>
<td>PA Net Pain</td>
<td>Allocated to INT (n = 112)</td>
<td>Received INT (n = 111)</td>
<td>No INT (n = 1)</td>
</tr>
<tr>
<td>PA MHL</td>
<td>Allocated to INT (n = 110)</td>
<td>Received INT (n = 110)</td>
<td>No INT (n = 0)</td>
</tr>
<tr>
<td>PA Pain</td>
<td>Allocated to INT (n = 110)</td>
<td>Received INT (n = 110)</td>
<td>No INT (n = 0)</td>
</tr>
<tr>
<td>PA Net MHL</td>
<td>Allocated to INT (n = 111)</td>
<td>Received INT (n = 111)</td>
<td>No INT (n = 0)</td>
</tr>
<tr>
<td>PA Net Pain</td>
<td>Allocated to INT (n = 111)</td>
<td>Received INT (n = 111)</td>
<td>No INT (n = 0)</td>
</tr>
</tbody>
</table>

Total not meeting inclusion criteria (n = 14,684)
- Low BMI Score (n = 10,416)
- High physical activity level (n = 511)
- Taking vitamin B complex supplements (n = 1,155)
- Health-related reasons (n = 1,238)
- Informed consent not received (n = 267)
- Low blood folate and vitamin B12 levels (n = 317)
- Declined to participate (n = 8,749)
- Returned late (n = 19)

Reasons for non-eligible (n = 3): non-compliant, moved, refused tablets.
participants in FA + vitamin B-12 and placebo groups who completed the 24-mo interview (chi-square = 0.6, P = 0.420).

Oral FA + vitamin B-12 supplementation

In comparison with placebo, the FA + vitamin B-12 group showed a significant increase in concentrations of folate (an increase of 65.97% from 573 to 951 nmol/L; t681 = 12.0, P < 0.001; the placebo group had an increase of 1.97% from 557 to 568 nmol/L) and in vitamin B-12 (an increase of 55.74% from 305 to 475 nmol/L; t681 = 14.5, P < 0.001; the placebo group had an increase of 4.21% from 285 to 297 nmol/L) over the 24-mo period. Homocysteine increased significantly less in the FA + vitamin B-12 group (from 9.6 to 10.4 μmol/L; an increase of 8.33%) than in the placebo group (from 9.8 to 12.0; an increase of 22.45%; t649 = -5.6, P < 0.001).

Cognitive functioning

Variable estimates from the final models for the TICS-M total cognitive score, TICS-M immediate memory, and TICS-M delayed recall are presented in Table 2, with all of the models taking into account baseline homocysteine concentrations and depression levels. In addition to analyzing the data for the TICS-M total cognitive score, separate mixed-model repeated-measures ANOVAs were used for each TICS-M subscale to determine whether there were particular subscales that may further explain in which aspect of cognitive functioning any significant effect may have occurred. Findings for all other cognitive measures—i.e., TICS-M orientation, TICS-M attention/calculations, TICS-M semantic memory, BTACT processing speed, and IQCODE informant-reported cognitive functioning—were not significant, and results are not shown.

Omnibus tests of time by intervention effects on TICS-M total cognitive score, TICS-M immediate memory, and TICS-M delayed recall from baseline to the completion of the intervention at 24 mo were significant (F2,788.4 = 5.26, P = 0.005). The FA + vitamin B-12 group had a significantly greater increase in TICS-M total scores from baseline to 24 mo than did the placebo group (P = 0.032, effect size = 0.17). The effect was not significant at 12 mo (P = 0.283). The FA + vitamin B-12 group showed significantly greater increases in performance than did the placebo control in TICS-M immediate recall (P = 0.046, effect size = 0.15) and TICS-M delayed recall (P = 0.013, effect size = 0.18) from baseline to 24-mo assessment (Figure 2).

In addition to the intervention effects, baseline homocysteine concentrations and depression scores had important associations with cognitive performance over time. Elevated homocysteine concentrations at baseline were associated with poorer cognitive performance at 24 mo for TICS-M overall cognitive performance (t649 = -2.93, P = 0.004), immediate recall (t649 = -4.95, P = 0.001), and delayed recall (t649 = -3.45, P = 0.001). Similarly, initially higher depression scores (Patient Health Questionnaire—9) were predictive of lower cognitive performance at 24 mo, including TICS-M overall cognitive score (t687 = -2.43, P = 0.016), immediate recall (t687 = -2.28, P = 0.023), and orientation (t687 = -2.27, P = 0.024).

Subsidiary analyses were conducted to determine the role of baseline homocysteine and change in homocysteine scores on cognitive functioning scores. The model was reevaluated with the inclusion of a measure of the overall change in homocysteine from baseline to 24 mo as a covariate (for completers of the 24-mo interview, n = 595). In this analysis, after baseline homocysteine concentrations were accounted for, smaller increases in homocysteine were associated with significantly larger increases in TICS-M total score from baseline to 12 mo (t609 = -2.01, P = 0.044), but there was no effect at 24 mo (t610 = -0.99, P = 0.325).

DISCUSSION

In this study, oral FA (400 μg/d) + vitamin B-12 (100 μg/d) supplementation led to significantly greater improvements in overall cognitive functioning scores (TICS-M total score) at 24 mo than did the placebo condition (effect size Cohen’s d = 0.17, P = 0.032, with control for baseline covariates including depressive symptoms and concentrations of homocysteine, folate, and vitamin B-12). The FA + vitamin B-12 group had significantly greater improvements in both immediate (Cohen’s d = 0.15, P < 0.046) and delayed (Cohen’s d = 0.18, P < 0.013) recall from baseline to the completion of the intervention at 24 mo compared with the placebo group. No significant changes were evident for TICS-M orientation, attention/calculations, semantic memory, and BTACT processing speed informant-reported cognitive functioning.

Our findings are incongruent with some of the existing evidence that found no effect for FA and vitamin B-12 supplementation on cognitive performance (10, 13, 16, 39). Nonetheless, our study concurs with and extends previous evidence from both short- and long-term interventions (18, 40). For instance, one long-term intervention of 800 μg FA/d (36-mo duration) found benefits in memory and global function in healthy older adults with elevated plasma total homocysteine concentrations (40).
Even though the findings for FA + vitamin B-12 supplementation were in keeping with some previous trials (18, 40), a number of our findings require explanation. First, the effect was evident for short- and long-term memory, but not processing speed, which may be explained by the relation between folate and hippocampal function. Evidence from RCTs (18, 40) and animal

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>FA + vitamin B-12 (n = 447)</th>
<th>Placebo (n = 453)</th>
<th>P value</th>
<th>Total (n = 900)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>65.92 ± 4.30²</td>
<td>65.97 ± 4.18</td>
<td>0.861</td>
<td>65.94 ± 4.24</td>
</tr>
<tr>
<td>Men [n (%)]</td>
<td>181 (40.5)</td>
<td>177 (39.1)</td>
<td>0.664</td>
<td>358 (39.8)</td>
</tr>
<tr>
<td>Marital status [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married/de facto</td>
<td>286 (64.0)</td>
<td>278 (61.4)</td>
<td>0.119</td>
<td>564 (62.2)</td>
</tr>
<tr>
<td>Separated/divorced</td>
<td>86 (19.2)</td>
<td>75 (16.6)</td>
<td>0.196</td>
<td>161 (17.2)</td>
</tr>
<tr>
<td>Widowed</td>
<td>51 (11.4)</td>
<td>59 (13.0)</td>
<td>0.119</td>
<td>110 (12.2)</td>
</tr>
<tr>
<td>Never married</td>
<td>24 (5.4)</td>
<td>41 (9.1)</td>
<td>0.041</td>
<td>65 (7.2)</td>
</tr>
<tr>
<td>Employment status [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not in the labor force</td>
<td>270 (61.4)</td>
<td>289 (64.9)</td>
<td>0.117</td>
<td>569 (62.3)</td>
</tr>
<tr>
<td>Employed, full-time</td>
<td>72 (16.4)</td>
<td>50 (11.2)</td>
<td>0.196</td>
<td>130 (14.4)</td>
</tr>
<tr>
<td>Employed part-time</td>
<td>90 (20.5)</td>
<td>91 (20.4)</td>
<td>0.983</td>
<td>163 (18.1)</td>
</tr>
<tr>
<td>Unemployed</td>
<td>8 (1.8)</td>
<td>15 (3.4)</td>
<td>0.117</td>
<td>11 (1.2)</td>
</tr>
<tr>
<td>Education (y)</td>
<td>13.77 ± 2.71</td>
<td>13.92 ± 2.86</td>
<td>0.407</td>
<td>13.84 ± 2.78</td>
</tr>
<tr>
<td>Depression</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K10 score</td>
<td>17.28 ± 5.36</td>
<td>17.56 ± 5.15</td>
<td>0.430</td>
<td>17.42 ± 5.25</td>
</tr>
<tr>
<td>PHQ-9 depression score</td>
<td>5.37 ± 4.21</td>
<td>5.58 ± 4.27</td>
<td>0.448</td>
<td>5.47 ± 4.24</td>
</tr>
<tr>
<td>0–9 [n (%)]</td>
<td>380 (85.0)</td>
<td>373 (82.5)</td>
<td>0.753</td>
<td>753 (83.8)</td>
</tr>
<tr>
<td>10–14 [n (%)]</td>
<td>48 (10.7)</td>
<td>59 (13.1)</td>
<td>0.107</td>
<td>117 (13.1)</td>
</tr>
<tr>
<td>15–19 [n (%)]</td>
<td>14 (3.1)</td>
<td>19 (4.2)</td>
<td>0.333</td>
<td>33 (3.7)</td>
</tr>
<tr>
<td>≥20 [n (%)]</td>
<td>5 (1.1)</td>
<td>1 (0.2)</td>
<td>0.333</td>
<td>6 (0.7)</td>
</tr>
<tr>
<td>No. of medical conditions</td>
<td>1.53 ± 1.19</td>
<td>1.48 ± 1.13</td>
<td>0.507</td>
<td>1.51 ± 1.16</td>
</tr>
<tr>
<td>Have vascular condition</td>
<td>0.71 ± 0.45</td>
<td>0.75 ± 0.43</td>
<td>0.162</td>
<td>0.73 ± 0.44</td>
</tr>
<tr>
<td>History of [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain tumor</td>
<td>3 (0.7)</td>
<td>4 (0.9)</td>
<td>0.718</td>
<td>7 (0.8)</td>
</tr>
<tr>
<td>Stroke</td>
<td>9 (2.0)</td>
<td>9 (2.0)</td>
<td>0.977</td>
<td>18 (2.0)</td>
</tr>
<tr>
<td>Mini-stroke</td>
<td>22 (4.9)</td>
<td>34 (7.5)</td>
<td>0.112</td>
<td>56 (6.2)</td>
</tr>
<tr>
<td>Serious head injury</td>
<td>47 (10.5)</td>
<td>55 (12.1)</td>
<td>0.420</td>
<td>102 (11.3)</td>
</tr>
<tr>
<td>Heart problem</td>
<td>79 (17.7)</td>
<td>85 (18.8)</td>
<td>0.683</td>
<td>164 (18.2)</td>
</tr>
<tr>
<td>Heart attack</td>
<td>13 (2.9)</td>
<td>15 (3.3)</td>
<td>0.810</td>
<td>28 (3.1)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>206 (46.1)</td>
<td>231 (51.0)</td>
<td>0.140</td>
<td>437 (48.6)</td>
</tr>
<tr>
<td>Use of hypertension medication [n (%)]</td>
<td>172 (38.5)</td>
<td>196 (43.3)</td>
<td>0.539</td>
<td>368 (40.9)</td>
</tr>
<tr>
<td>Faintness/dizziness standing up [n (%)]</td>
<td>165 (36.9)</td>
<td>180 (39.7)</td>
<td>0.398</td>
<td>345 (38.3)</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>5.24 ± 0.99</td>
<td>5.23 ± 0.94</td>
<td>0.932</td>
<td>5.24 ± 0.96</td>
</tr>
<tr>
<td>Homocysteine in plasma (µmol/L)</td>
<td>9.59 ± 2.56</td>
<td>9.81 ± 2.78</td>
<td>0.215</td>
<td>9.70 ± 2.68</td>
</tr>
<tr>
<td>Red blood cell folate (µmol/L)</td>
<td>572.54 ± 266.32</td>
<td>557.09 ± 277.50</td>
<td>0.395</td>
<td>564.77 ± 271.96</td>
</tr>
<tr>
<td>Serum vitamin B-12 (nmol/L)</td>
<td>305.32 ± 151.05</td>
<td>285.27 ± 105.77</td>
<td>0.021</td>
<td>295.24 ± 130.58</td>
</tr>
<tr>
<td>IPAQ MET (min/wk)</td>
<td>1705.99 ± 1791.81</td>
<td>1651.33 ± 1830.17</td>
<td>0.651</td>
<td>1678.45 ± 1810.44</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cognitive function</th>
<th>FA + vitamin B-12 (n = 447)</th>
<th>Placebo (n = 453)</th>
<th>P value</th>
<th>Total (n = 900)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTACT processing speed</td>
<td>38.39 ± 10.64</td>
<td>37.95 ± 10.78</td>
<td>0.532</td>
<td>38.17 ± 10.71</td>
</tr>
<tr>
<td>IQCODE score</td>
<td>3.06 ± 0.25</td>
<td>3.03 ± 0.26</td>
<td>0.223</td>
<td>3.05 ± 0.26</td>
</tr>
<tr>
<td>TICS-M total score</td>
<td>26.42 ± 3.87</td>
<td>26.67 ± 3.69</td>
<td>0.316</td>
<td>26.55 ± 3.78</td>
</tr>
<tr>
<td>TICS-M orientation</td>
<td>6.65 ± 0.69</td>
<td>6.68 ± 0.62</td>
<td>0.415</td>
<td>6.66 ± 0.65</td>
</tr>
<tr>
<td>TICS-M immediate recall</td>
<td>5.16 ± 1.54</td>
<td>5.23 ± 1.59</td>
<td>0.500</td>
<td>5.19 ± 1.56</td>
</tr>
<tr>
<td>TICS-M delayed recall</td>
<td>3.75 ± 1.63</td>
<td>3.89 ± 1.63</td>
<td>0.203</td>
<td>3.82 ± 1.63</td>
</tr>
</tbody>
</table>

¹ Comparisons for each intervention arm were averaged over other arms. P values were based on F statistics for continuous variables and on chi-square statistics for categorical variables. Baseline data were collected from January to August 2006. BTACT, Brief Test of Adult Cognition by Telephone; FA, folic acid; IPAQ MET, International Physical Activity Questionnaire metabolic equivalent task; IQCODE, Informant Questionnaire on Cognitive Decline in the Elderly; K10, Kessler Distress 10–Scale; PHQ-9, Patient Health Questionnaire–9; TICS-M, Telephone Interview of Cognitive Status–Modified.

² Mean ± SD (all such values).
³ PHQ severity categories include the following: minimal-mild (0–9), moderate (10–14), moderately severe (15–19), and severe (≥20) (36).
⁴ Those who received FA + vitamin B-12 had higher concentrations of serum vitamin B-12 than did those in the placebo group (F1,898 = 5.33, P = 0.021).
models (41) suggests that FA + vitamin B-12 supplementation may have a positive impact on global cognitive functioning and specifically on performance on memory tasks (42). In terms of memory performance, a proposed mechanism has been posited that a reduction in BDNF may be related to oxidative stress, which reduces BDNF mRNA expression, and in turn impairs the promotion of hippocampal neurons (43, 44). Elevated homocysteine concentrations may induce oxidative stress and related neurotoxicity,
which possibly lead to reduced BDNF concentrations and impaired BDNF pathways in the hippocampus, that may impair memory consolidation (41, 44). These few possible mechanisms in the relation between folate concentrations and memory performance may explain our lack of effect found for processing speed. FA + vitamin B-12 supplementation may have greater effect on processes within the hippocampus and its functioning than on other processes or areas that are more connected to processing speed. Changes in processing speed may be associated with structural declines in neural networks in the prefrontal cortical and cerebella regions (45). In addition, processing speed involves the coordination of numerous activities (eg, perception, decision making) and processing speed tasks (45): hence, the complex interaction between numerous brain networks and processing speed as a construct may be too diffuse to benefit from changes related to FA + vitamin B-12 supplementation.
The significant effect of FA + vitamin B-12 supplementation occurred in the later stage of the intervention, ie, at 24 mo. It is possible that the effects of FA + vitamin B-12 supplementation are long term and operate by reducing vascular and other metabolic risk factors for cognitive impairment (10, 46). We found a modest association between larger increases in homocysteine concentrations and smaller increases in cognitive performance, suggesting that the effect is related to homocysteine concentrations. An alternative explanation, supported by evidence from animal models, is that folate deficiency and elevated homocysteine concentrations impair DNA repair in hippocampal neurons and sensitize them to amyloidial toxicity (47). The latter mechanism may be plausible because a recent study showed a higher retention of Pittsburgh Compound-B, a selective imaging ligand for β-amyloid in the brain, in depressive patients compared with controls (48). Furthermore, folate and vitamin B-12 may selectively benefit the hippocampus, because this is one of the unique regions in the brain in which cell renewal and DNA replication occurs and therefore may have a higher dependence on these vitamins that are essential for nucleotide synthesis (49).

Despite the lack of clarity regarding the mechanism of FA + vitamin B-12 supplementation in cognitive performance and decline, we found that 400 μg FA + 100 μg vitamin B-12/d is potentially an effective long-term intervention for minimizing cognitive decline in a dose that can be recommended as a dietary supplement.

Finally, the overall cognitive functioning scores (Cohen’s d = 0.31, P < 0.001), as well as immediate (P = 0.034) and delayed (P < 0.001) recall, improved over the 2-y period. This was likely due to practice effects. The fact that greater improvement in performance occurred with FA + vitamin B-12 supplementation is consistent with suggestions that those with a capacity to learn may be less prone to experience cognitive decline (50).

**Strengths and limitations**

In interpreting the benefits found in public health terms, it is important to bear in mind that we received 23.19% of surveys originally sent out into the community, and only 3.7% of those who returned their surveys met the selection criteria to participate in the intervention, which may create uncertainty about generalizing from our findings. This relatively small group in comparison to the total of surveys returned was a consequence of cell renewal and DNA replication occurs and therefore may have a higher dependence on these vitamins that are essential for nucleotide synthesis. (49).

Despite the lack of clarity regarding the mechanism of FA + vitamin B-12 supplementation in cognitive performance and decline, we found that 400 μg FA + 100 μg vitamin B-12/d is potentially an effective long-term intervention for minimizing cognitive decline in a dose that can be recommended as a dietary supplement.

Finally, the overall cognitive functioning scores (Cohen’s d = 0.31, P < 0.001), as well as immediate (P = 0.034) and delayed (P < 0.001) recall, improved over the 2-y period. This was likely due to practice effects. The fact that greater improvement in performance occurred with FA + vitamin B-12 supplementation is consistent with suggestions that those with a capacity to learn may be less prone to experience cognitive decline (50).

**Conclusions and further research**

Our findings suggest that there may be a role for combined FA + vitamin B-12 in lowering the risk of cognitive decline. Such an intervention is inexpensive, and at the population level the preventive effect may be considerable (10), at least among people with subsyndromal depressive disorders. The prospect of using dietary supplementations of FA and vitamin B-12 to prevent cognitive decline appears promising. More studies are needed to determine whether the benefits of FA and vitamin B-12 supplementation found in this trial could be replicated in other populations of older adults with increased risk of developing significant cognitive impairment.

We thank the participants of the Beyond Ageing Project for their involvement and enthusiasm. We gratefully acknowledge the support of Elizabeth Parkes and the telephone interviewing team; the research assistance from Amanda George; and the administrative support from Carmel Poyser, Kim Pullen, Trish Jacomb, and Karen Maxwell; and input in the research conception and design from Kaarin Anstey, Mike Bird, and Kathy Griffiths.

The authors’ responsibilities were as follows—AFJ, IH, HC, and JGW: designed the study; JGW, DC, and HC: acquired the data; PJB and AJM: analyzed the data; JGW, HC, PJB, AJM, AFJ, and DC: drafted the manuscript; HC, JGW, AJM, PJB, AFJ, IH, MF, and MK: provided critical revision of the manuscript for important intellectual content; AFJ, HC, and IH: obtained funding; and JGW and HC: supervised the study. All authors had access to the data in the study and take responsibility for the integrity of the data and accuracy of the data analyses and final report. The sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, or approval of the manuscript. None of the authors had any conflicts of interest.

**REFERENCES**


9. Pullen, Trish Jacomb, and Karen Maxwell; and input in the research conception and design from Kaarin Anstey, Mike Bird, and Kathy Griffiths.


