Supplementary Methods

Further study protocol details

Via a telephone interview, respondents completed a health screening questionnaire relating to inclusion and exclusion criteria, and completed the TICS-M and a category fluency test (CERAD) [1,2]. Eligible participants were asked if they would agree to have two cranial MRI scans, one at the start and one two years later at the end of treatment, but it was emphasized that the scans were voluntary. Although the main outcome of the trial was atrophy rate, we allowed subjects that could not or did not wish to participate in the MRI part of the study to enter the trial in a separate arm: the intention was to evaluate recruiting strategies for such trials (not reported here) for subjects with MCI.

At the initial clinic visit, participants provided blood and urine samples and underwent a variety of cognitive tests (to be described separately), including the MMSE [3], and they completed the Geriatric Depression Scale (GDS) questionnaire [4]. Participants also had a simple test of their vibration sense in the ankle with a tuning fork. Participants were contacted by telephone at the following times after starting treatment: 3, 6, 12, 15 and 18 months to check compliance, ask about adverse events and to carry out a verbal memory test. After 24 months, participants returned to the clinic for examination, blood tests described in Table 1; the cognitive test battery was repeated as well. A final telephone assessment of adverse events and cognition was done at 27 months.

Placebo tablet

The placebo tablet was identical to the vitamin tablet (TrioBe Plus®) except for omission of the vitamins and the addition of iron oxide and ferrous sulphate (0.0055%) to give a colour to match the vitamin tablet.

Blood sampling and assays

At baseline and after 2 y, non-fasting blood samples were collected by venipuncture. Plasma tHcy, folate, cobalamin, holoTC, and total TC were determined as previously described [5]. TC saturation was calculated as a ratio of holoTC to total TC. Plasma cystathionine was determined by liquid chromatography tandem mass spectrometry [6].

Genomic DNA was extracted from blood using the Wizard DNA Purification Kit (Promega, Southampton, UK). The *MTHFR* 677C>T polymorphism (NCBI Entrez Gene 4524) and the *TCN2* 776C>G polymorphism (NCBI Entrez Gene 6948) were genotyped using the Amplifluor SNP Genotyping System (Chemicon, Watford, UK) [7], while *APOE* genotypes (NCBI Entrez Gene: 348) was determined using a one-stage PCR method [8].

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