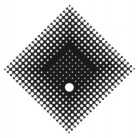
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Walking or vitamin B for cognition in older adults with mild cognitive impairment? A randomized controlled trial

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ABSTRACT

Objective: To examine the effects of aerobic exercise or vitamin B supplementation on cognitive function in older adults with mild cognitive impairment (MCI).
Design: Randomized placebo-controlled trial.
Setting: General community.
Participants: community-dwelling adults aged 70-80 with MCI.
Interventions: The 152 participants were randomly assigned to two interventions: 1) a twice-weekly, group-based, moderate-intensity walking program (WP, n=77) or a low-intensity placebo activity program (n=75) for one year; and 2) daily vitamin pill containing 5 mg folic acid, 0.4 mg vitamin B12, 50 mg vitamin B6 (FA/B12/B6, n=78) or placebo-pill (n=74) for one year.
Outcome measures: Cognitive function, measured with neuropsychological tests at baseline and after six and 12 months.
Results: Median session attendance to the exercise programs (25th-75th percentile) was 63 (2-81) percent and median compliance with taking pills (25th-75th percentile) was 100 (99-100) percent. Gender was an effect-modifier. Intention-to-treat analysis revealed no main intervention effect for either intervention. In women in the WP, attention (Stroop combination task) improved by 0.3 seconds (p=0.04) and memory (auditory verbal learning test) by 0.04 words (p=0.06) with each percent increase in session attendance. In men attending at least 75 percent of the sessions, the WP improved memory (beta [95%CI]= 1.5 [0.1; 3.0] words).
Conclusion: The walking program and/or FA/B12/B6 supplementation were not effective in improving cognition within one year. The walking program, however, was efficacious in improving memory in men and memory and attention in women with better adherence.
Trial Registration: International Standard Randomized Controlled Trial Number Register, 19227688, http://www.controlled-trials.com/isrctn/.

INTRODUCTION

Cognitive impairment is an important public health concern, since the number of elderly people with age-related health problems, such as cognitive decline and dementia, is growing considerably. Research on potentially effective interventions for preventing the progression of cognitive impairment is therefore warranted, and physical exercise and vitamin B supplementation are promising and relatively inexpensive interventions.

Regular exercise is associated with better cognitive function and delayed onset of Alzheimer's disease,[1-4] and has been shown to improve cognitive function in cognitively healthy and demented older adults.[5;6] The specific mechanisms of exercise beneficially affecting cognitive performance in subjects in different stages of cognitive decline are not yet known. A recent study of 59 sedentary adults aged 60-79 years has shown that six months of aerobic training increased brain volume in regions of the brain associated with age-related decline in cognition, compared with non-aerobic training.[7] Improvement of vascularisation and increased blood flow to the brain are other possible pathways, which may be promoted by improving aerobic fitness.[8;9]

Vitamin B supplementation may also affect cognitive function through its effect on homocysteine,[10] which is associated with impaired cognition.[11-16] There have however been few methodologically sound trials on the effect of vitamin B supplementation on cognition.[17-19] Vitamin B supplementation may affect cognitive performance via two mechanisms.[20] First, vitamin B is needed to transform homocysteine into methionine, which is an essential amino acid for the central nervous system. Lack of methionine results in disorders in neurological and psychological status. Second, vitamin B may beneficially influence homocysteine concentrations, resulting in structural vascular changes in the brain.

This paper describes the effects of two interventions on cognitive performance in a sample of community-dwelling older adults with mild cognitive impairment (MCI). MCI is a potential transitional stage between normal cognitive function and Alzheimer's Disease, in which people experience memory loss to a greater extent than is expected for age and education, but do not meet criteria for Alzheimer's disease.[21] The interventions were: 1) one year of a moderate intensity walking program designed to improve aerobic fitness; and 2) one year of daily supplementation with folic acid, vitamins B12 and B6. It was hypothesized that both interventions would beneficially influence cognitive performance.

METHODS

Study design

The study was a double-blind randomized controlled trial (RCT). A two by two factorial design was used to compare the effects of: 1) a walking program with a placebo activity program; and 2) vitamin B supplementation with placebo-supplementation in a single sample. Thus, half of the subjects in the walking program got vitamin B supplements and the other half got placebo supplements. This was also the case within the placebo activity program. This design allows two hypotheses to be tested at the same time, by randomizing subjects to two interventions and adjusting the results from each intervention for the effects of the other.[22;23] The medical ethics committee approved the study-protocol.[24] All participants gave written informed consent.

Participants
All community-dwelling inhabitants aged 70 to 80 years in a Dutch town (n=5491) were sent an invitation letter in September 2003. Among those willing to participate, inclusion criteria were checked using a two-step screening consisting of a postal questionnaire and a telephone interview.[25] The operational criteria for MCI and additional inclusion criteria for the trial are described in Table 1.

Table 1 Inclusion and exclusion criteria for participation in the trial

Operationalisation of Petersen criteria for MCI (1-5) and additional inclusion criteria for the RCT (6-12)
1. Memory complaints (answer 'yes' to question 'do you have memory complaints', or at least twice answering 'sometimes' on the Strawbridge cognition scale [26])
2. Objective memory impairment (10 WLT[27] delayed recall ≤ 5 and percentage savings ≤ 100)
3. Normal general cognitive function (TICS[28] ≥ 19 and MMSE[29] ≥ 24)
4. Intact daily functioning (no report of disability in activities of daily living on GARS-scale[30], except on the item 'taking care of feet and toe nails')
5. Absence of dementia (TICS[28] ≥ 19 and MMSE[29] ≥ 24)
6. Being able to perform moderate intensity physical activity, without making use of walking devices, e.g. a rollator or a walking frame
7. Not using vitamin supplements/ vitamin injections/ drinks with folic acid, vitamins B12 and B6, comparable to the vitamin supplement given in the intervention
8. Not suffering from epilepsy, multiple sclerosis, Parkinson's disease, kidney disorder requiring haemodialysis, psychiatric impairment
9. Not suffering from depression as measured by the GDS[31](cut off ≤ 5)
10. Not using medication for rheumatoid arthritis or psoriasis which interfered with the vitamin supplement
11. No alcohol abuse (men < 21 drinks a week, women < 15 drinks a week)
12. Not currently living in a nursing home or on a waiting list for a nursing home

MCI= Mild Cognitive Impairment; RCT= Randomized Controlled Trial; 10 WLT= 10 Word Learning Test; TICS= Telephone Interview for Cognitive Status; MMSE= Mini Mental State Examination; GARS= Groningen Activity Restriction Scale; GDS=Geriatric Depression Scale

Randomization and blinding
After the baseline interview, participants were randomized using the option 'random sample of cases' in SPSS. Randomization was stratified for physical activity level assessed by the LASA physical activity questionnaire.[32] Participants were randomized to: 1) walking program or placebo activity program; then within each activity program subjects were randomized to: 2) vitamin B supplementation or placebo supplementation. Participants and exercise instructors were blinded to group allocation by being left unaware of which exercise program was supposed to be effective. The pills were coded as *A* or *B* by the manufacturer. The key was decoded after data-analysis. All cognitive outcome measures were assessed by trained examiners, who were also blinded to group allocation.

Interventions

Walking program (WP) and Placebo Activity program (PAP)

Both the WP and the PAP were group-based and lasted one year (June 2004 – June 2005). Trained instructors supervised exercise sessions twice-weekly for one hour. Adherence was defined as the percentage of attended sessions.

The WP was based on an existing aerobic walking program designed to improve aerobic fitness,[33] and intensity was moderate (> 3 metabolic equivalents). Each session consisted of a warm-up, moderate-intensity walking exercises and cool-down. The instructors monitored exercise intensity during all sessions subjectively – e.g. participants were still able to talk, but also showed signs of moderate intensity activity, such as increased breathing frequency. Walking-pace and distance increased gradually during the program. Sessions took place outdoors in municipal parks.

The PAP, developed by experienced exercise instructors, consisted of an introduction, low-intensity (<3 metabolic equivalents) non-aerobic group exercises and a closing. Sessions included relaxation, activities of daily living, balance, flexibility and postural exercises. The PAP was carried out in community centres.

Vitamin B supplementation (FA/B12/B6) and placebo supplementation

Participants randomized to FA/B12/B6-supplementation received one pill daily, containing 5 mg vitamin B11 (Folic Acid), 0.4 mg vitamin B12 (Cyanocobalamin) and 50 mg vitamin B6 (Pyridoxine-hydrochloride) for a year. These vitamin supplements are available on prescription in The Netherlands. For the purpose of this study, the package and vitamin pills could not be identified as an existing supplement. Participants in the control group received an identical-looking placebo-pill. Pills were packed in one-week blister packs labelled for each day of the week. Compliance was verified by counting pills in returned blister packs.

Measures

Measurement of baseline variables is described in detail elsewhere.[24] Cognitive measures were collected at baseline and after six and 12 months during a standardized interview. The Mini Mental State Examination (MMSE) was used to measure general cognitive function for descriptive purposes. The maximum score is 30, and a score below 24 was considered abnormal.[29] Four other tests were administered to assess different aspects of cognition:

- Memory by the Auditory Verbal Learning Test (AVLT) (immediate and delayed recall; maximum scores 75 and 15 words, respectively).[34]
- Executive function by the verbal fluency test (VFT). Participants were asked to name words starting with a particular letter during a one-minute period. In each administration of the test, three letters were given. The score was the total number of named words.[35]
- Information processing speed by the Digit Symbol Substitution Test (DSST). The participant was asked to draw symbols corresponding to nine digits below numbered boxes during a 90-second period. The score was the number of correctly drawn symbols.[36]
- Attention by the Abridged Stroop Colour Word Test (SCWT-A). The SCWT-A consists of three tasks; 1) reading eight rows of five written colours; 2) naming the colours of eight rows of five red, green, blue or yellow coloured rectangles; 3) naming the colour of ink for the words *red*, *green*, *blue* or *yellow*. The score was the time needed to complete each task.[37]

Statistical analysis

Sample size

Since participants were selected on the basis of memory complaints and impairments, memory was considered the most important cognitive outcome in our study. Therefore, sample size calculations were based on AVLT data.[21] The average difference between subjects with MCI and mild Alzheimer's disease and subjects with MCI and Alzheimer's disease for immediate recall was five words. On the basis of this difference, it was estimated that 136 participants would be required ($\alpha = 0.05$ and $\beta = 0.80$). To allow for a drop-out of 25 percent, the final sample size was 179 participants.

Data analysis

Differences in baseline characteristics between intervention and control groups (e.g. WP versus PAP and FA/B12/B6 versus placebo) were tested using independent t-tests, Mann Whitney U tests and chi-square tests. For both interventions, data were analyzed on a modified intention-to-treat basis, including participants with at least one post-baseline assessment. Longitudinal regression analysis was used to assess changes in each outcome measure. The two follow-up measurements were defined as dependent variable and multi-level analysis with two levels was used, 1) time of follow-up measurement (values corresponding with performance after six and 12 months intervention); 2) individual. Reported regression coefficients indicate differences between WP and PAP, or FA/B12/B6-supplementation and placebo-supplementation. Data were analyzed using crude and adjusted models. In the crude models, the independent variables were exercise intervention, vitamin intervention and baseline performance. In the adjusted models, education, baseline activity level, baseline vitamin status, adherence to the exercise program and compliance with the supplementation were added as covariates. Effect modification by gender was checked. Because gender was an effect modifier in four of seven outcome measures, analyses for all outcomes were stratified by gender.

Since adherence with the WP and the PAP was sub-optimal, a post-hoc analysis was performed to check for effect modification by session attendance. Finally, a per protocol analysis, including only participants who attended at least 75 percent of the exercise sessions was performed. This cut-off point is in concordance with previous exercise intervention studies in elderly people.[39;40] Data were analyzed using SPSS (release 12.0.2). A significance level of five percent was used for between-group comparisons and of ten percent for interaction terms.

RESULTS

Participant flow and characteristics

After baseline measurement, 179 participants were randomized. Twenty-seven of them were excluded from the analysis, because they only provided baseline data (Figure 1). Compared with the remaining 152 participants, a higher percentage of these 27 participants was married (71 versus 52 percent, p=0.05), and fewer were current smokers (0 versus 14 percent, p=0.04). No significant differences existed between measures of cognition. Baseline variables for men and women are shown in Table 2. Participants in the WP did not differ significantly from participants in the PAP. In the FA/B12/B6-supplementation group, a higher percentage of men had lower education and a higher percentage of women had intermediate/high education compared with the placebo-supplementation group.

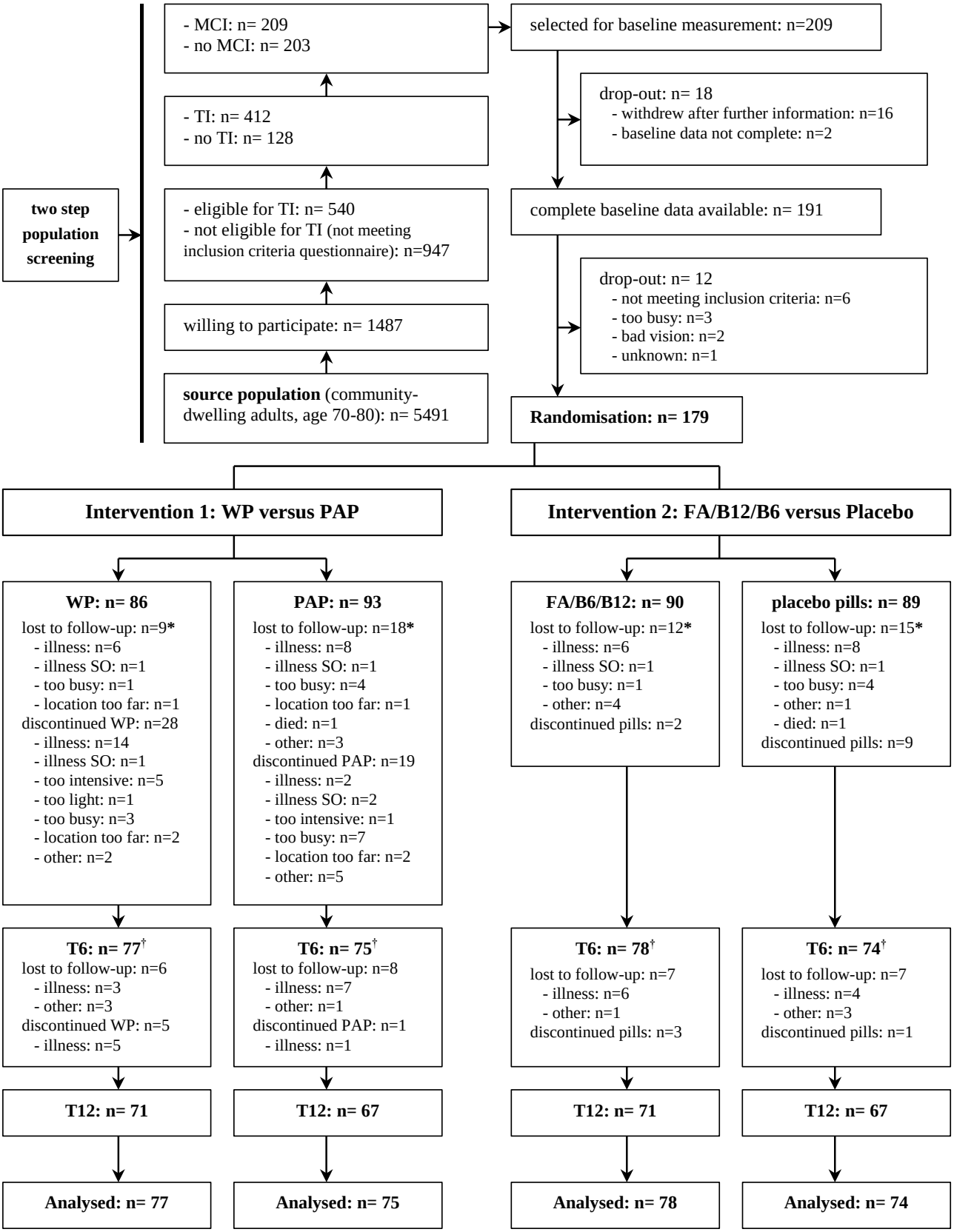
- MCI: n= 209
- no MCI: n= 203
selected for baseline measurement: n=209
drop-out: n= 18
- withdrew after further information: n=16
- baseline data not complete: n=2
- TI: n= 412
- no TI: n= 128
two step population screening
- eligible for TI: n= 540
- not eligible for TI (not meeting inclusion criteria questionnaire): n=947
complete baseline data available: n= 191
drop-out: n= 12
- not meeting inclusion criteria: n=6
- too busy: n=3
- bad vision: n=2
- unknown: n=1
willing to participate: n= 1487
source population (community-dwelling adults, age 70-80): n= 5491
Randomisation: n= 179
Intervention 1: WP versus PAP
Intervention 2: FA/B12/B6 versus Placebo
WP: n= 86
lost to follow-up: n=9*
- illness: n=6
- illness SO: n=1
- too busy: n=1
- location too far: n=1
discontinued WP: n=28
- illness: n=14
- illness SO: n=1
- too intensive: n=5
- too light: n=1
- too busy: n=3
- location too far: n=2
- other: n=2
PAP: n= 93
lost to follow-up: n=18*
- illness: n=8
- illness SO: n=1
- too busy: n=4
- location too far: n=1
- died: n=1
- other: n=3
discontinued PAP: n=19
- illness: n=2
- illness SO: n=2
- too intensive: n=1
- too busy: n=7
- location too far: n=2
- other: n=5
FA/B6/B12: n= 90
lost to follow-up: n=12*
- illness: n=6
- illness SO: n=1
- too busy: n=1
- other: n=4
discontinued pills: n=2
placebo pills: n= 89
lost to follow-up: n=15*
- illness: n=8
- illness SO: n=1
- too busy: n=4
- other: n=1
- died: n=1
discontinued pills: n=9
T6: n= 77†
lost to follow-up: n=6
- illness: n=3
- other: n=3
discontinued WP: n=5
- illness: n=5
T6: n= 75†
lost to follow-up: n=8
- illness: n=7
- other: n=1
discontinued PAP: n=1
- illness: n=1
T6: n= 78†
lost to follow-up: n=7
- illness: n=6
- other: n=1
discontinued pills: n=3
T6: n= 74†
lost to follow-up: n=7
- illness: n=4
- other: n=3
discontinued pills: n=1
T12: n= 71
T12: n= 67
T12: n= 71
T12: n= 67
Analysed: n= 77
Analysed: n= 75
Analysed: n= 78
Analysed: n= 74

Figure 1 Flow Chart
TI= Telephone Interview; MCI= Mild Cognitive Impairment; WP= Walking Program; PAP= Placebo Activity Program; FA/B12/B6= Folic Acid, vitamins B12 and B6; SO= Significant Other; T6= Follow-up after 6 months; T12= Follow-up after 12 months; * Excluded from analysis (only baseline data available); † Included in analysis

Table 2 Baseline characteristics of the study population by intervention group (n=152)

	Walking Program vs. Placebo Activity Program				**FA/B12/B6 vs. placebo pills**			
	Men		Women		*Men*		Women	
	WP (n=37)	*PAP (n=48)*	WP (n=40)	PAP (n=27)	*vit B (n=44)*	*placebo (n=41)*	vit B (n=34)	placebo (n=33)
Age (Mean (SD))	*74 (2.7)*	*75 (2.8)*	76 (2.9)	75 (2.9)	*75 (2.7)*	*74 (2.9)*	76 (2.9)	76 (2.9)
MMSE (Median (25th; 75th ‰))	*29 (28; 29)*	*29 (28; 29)*	29 (28; 29)	29 (28; 30)	*28 (28; 29)*	*29 (28; 29)*	29 (29; 30)	29 (28; 30)
% low/ intermediate/ high education [a]	*57/ 16/ 27*	*42/ 35/ 23*	64/28/8	70/ 19/ 11	*61/ 21/ 18 **	*34/ 34/ 32*	52/ 33/ 15 *	82/ 15/ 3
% living with partner	*87*	*85*	50	56	*82*	*90*	53	52
PA, min/day (Median (25th; 75th ‰)) [b]	*79 (63; 137)*	*79 (36; 124)*	53 (42; 80)	48 (33; 110)	*77 (55; 125)*	*81 (35; 125)*	55 (41; 89)	51 (33; 80)
% deficient FA/B12/B6 [c]	*35/ 5/ 0*	*42/ 6/ 0*	55/ 10/ 0	59/ 11/ 0	*39/ 7/ 0*	*39 / 5/ 0*	62/ 12/ 0	52/ 9/ 0
% hyperhomocysteinemia [d]	*27*	*33*	20	15	*32*	*29*	21	15
% hypertension [e]	*19*	*32*	8	19	*30*	*22*	18	7
BMI, kg/m2 (Mean (SD))	*26.5 (3.4)*	*26.9 (3.1)*	26.7 (3.0)	28.3 (4.0)	*27 (3.4)*	*26.5 (3.0)*	27.5 (3.9)	27.5 (3.0)
% smokers	*11*	*15*	15	15	*18*	*7*	15	16

WP= Walking Program; PAP= Placebo Activity Program; FA/B12/B6= Folic Acid, vitamins B12 and B6; MMSE= Mini Mental State Examination; PA= Physical Activity; BMI= Body Mass Index; [a] *low*= no education, primary education, lower vocational training, *intermediate*= intermediate level secondary education, intermediate vocational training, *high*= higher level secondary education, higher vocational training, university training; [b] ≥ 3.0 metabolic equivalents; [c] cut off points: FA red blood cell < 305 nmol/L or FA plasma <6,3 nmol/L, B12 ≤ 150 pmol/L, B6 < 20 nmol/L; [d] homocysteine > 14 mmol/L; [e] hypertension= diastole ≥ 90 and systole ≥ 160;
* Significant difference between FA/B12/B6 and placebo-supplementation ($p<0.05$)

Adherence and compliance
Thirty participants, 19 men and 11 women, did not attend a single session, but were included in the intention-to-treat analysis. Median adherence (25^{th} – 75^{th} percentile) to both exercise programs, including these participants, was 63 (2-81) percent and did not differ between groups. At baseline, adherent men attending at least 75 percent of the sessions (n=33) were more often living with a partner (82% versus 65%, p=0.03) and reported to be less physically active than non-adherent men (n=52), (median [25^{th} -75^{th} percentile] was 64 [32-82] versus 87 [34-139] minutes/day, p=0.04). Only 18 women attended at least 75 percent of the sessions. The most frequent reasons for drop-out from the exercise programs after the start were health-related problems. No adverse events of the WP or PAP were reported.

Median compliance (25^{th} –75^{th} percentile) with the (vitamin) supplementation was 100 (99-100) percent. Two participants stopped taking vitamin FA/B12/B6-pills after reporting adverse side effects, i.e. sleep problems and increased forgetfulness; one participant discontinued the placebo-pills after reporting not feeling well.

Intention-to-treat analysis
In the intention to treat analysis (Table 3), no significant main effect of walking or FA/B12/B6-supplementation was found in men. In women, a beneficial main intervention effect of FA/B12/B6-supplementation was found on the DSST (beta [95%CI]= 2.9 [0.6; 5.3] symbols, p=0.02). Appendix Tables 1 and 2 present cognitive performance at baseline and after six and 12 months intervention.

Per protocol analysis
In women, an interaction was observed between the exercise programs and adherence. With each percentage increase in session attendance, in the WP, the SCWT-A combination task performance improved by 0.3 seconds (p=0.04), and the AVLT delayed recall score improved by 0.04 words (p=0.06). No interaction was observed in men. However, in men attending at least 75 percent of the sessions (n=33), the WP beneficially affected AVLT delayed recall (beta [95%CI]= 1.5 [0.1; 3.0] words, p=0.04). The number of women attending at least 75 percent of the sessions (n=18) was insufficient to perform a reliable per protocol analysis.

Table 3 Effect of the two interventions on change in cognition (adjusted model)

	Walking Program vs. Placebo Activity Program				**FA/B12/B6 vs. placebo pills**			
	Men *(WP: n=37 vs. PAP : n=48)*		Women (WP: n=40 vs. PAP: n=27)		*Men* *(vit B: n=44 vs. placebo: n=41)*		Women (vit B: n=34 vs. placebo: n=33)	
Outcome (unit)	*Beta (95%CI)* [c]	*p-value*	Beta (95%CI) [c]	p-value	*Beta (95%CI)* [c]	*p-value*	Beta (95%CI) [c]	p-value
AVLT 1-5 (words) [a]	*-1.4 (-4.1; 1.3)*	*0.31*	-0.6 (-3.8; 2.6)	0.72	*-2.2 (-5.1; 0.6)*	*0.12*	-0.1 (-3.4; 3.3)	0.97
AVLT 6 (words) [a]	*-0.2 (-1.0; 0.6)*	*0.58*	-0.4 (-1.6; 0.9)	0.56	*-0.5 (-1.3; 0.3)*	*0.25*	0.0 (-1.3; 1.3)	0.99
SCWT-A task 1 (s) [b]	*0.6 (-0.6; 1.8)*	*0.29*	-1.3 (-2.9; 0.3)	0.10	*0.0 (-1.3; 1.2)*	*0.98*	-0.9 (-2.6; 0.7)	0.27
SCWT-A task 2 (s) [b]	*-1.0 (-2.2; 0.2)*	*0.10*	0.6 (-0.9; 2.1)	0.46	*0.1 (-1.1; 1.4)*	*0.84*	-0.7 (-2.2; 0.9)	0.39
SCWT-A task 3 (s) [b]	*0.9 (-6.8; 8.7)*	*0.81*	-4.1 (-13.3; 5.2)	0.38	*3.1 (-5.1; 11.3)*	*0.45*	-1.1 (-10.8; 8.7)	0.83
DSST (symbols) [a]	*0.9 (-1.2; 3.0)*	*0.40*	0.8 (-1.4; 3.1)	0.47	*-1.4 (-3.7; 0.8)*	*0.20*	2.9 (0.6; 5.3)	0.02*
VFT (words) [a]	*-1.7 (-4.4; 1.0)*	*0.21*	-1.4 (-4.8; 2.0)	0.42	*0.8 (-2.0; 3.6)*	*0.58*	-1.0 (-4.5; 2.6)	0.59

WP= Walking Program; PAP = Placebo Activity Program; FA/B12/B6= Folic Acid, vitamins B12 and B6; AVLT= Auditory Verbal Learning Test; SCWT-A= Stroop Colour Word Test- Abridged; s= seconds; DSST= Digit Symbol Substitution Test; VFT= Verbal Fluency Test; [a] Higher score indicates better performance; [b] Lower score indicates better performance; [c] the beta's (95 % CI) indicate the difference in outcome between the walking program and the placebo activity program or between the vitamin pills and the placebo-pills: the unit is the unit of the outcome measure; * Significant difference between FA/B12/B6 and placebo

Appendix Table 1 Cognitive test performance at baseline and after six and 12 months in the walking program and the placebo activity program (mean (SD), unless indicated otherwise)

	Men in WP			***Men in PAP***			**Women in WP**			**Women in PAP**		
Outcome (unit)	*T0 (n=37)*	*T6 (n=37)*	*T12 (n=36)*	*T0 (n=48)*	*T6 (n=48*	*T12 (n=45)*	T0 (n=40)	T6 (n=40)	T12 (n=35)	T0 (n=27)	T6 (n=27)	T12 (n=22)
MMSE (points) [a, c]	*29 (28; 29)*	*28 (28; 29)*	*28 (27; 29)*	*29 (28; 29)*	*28 (27; 29)*	*28 (28; 29)*	29 (28; 29)	28 (28; 29)	29 (27; 29)	29 (28; 30)	29 (27; 30)	29 (27; 30)
AVLT 1-5 (words) [a]	*32.7 (7.8)*	*33.7 (10.8)*	*30.1 (9.4)*	*30.4 (6.9)*	*33.2 (9.1)*	*30.3 (9.0)*	34.6 (8.2)	34.2 (11.1)	32.1 (9.0)	34.3 (8.3)	34.7 (7.6)	33.0 (7.7)
AVLT 6 (words) [a]	*5.9 (2.5)*	*6.2 (3.0)*	*5.1 (2.9)*	*5.3 (2.3)*	*6.3 (3.1)*	*4.7 (2.6)*	6.3 (2.6)	6.2 (3.6)	5.6 (3.0)	6.0 (2.3)	6.5 (3.1)	5.5 (2.7)
SCWT-A task 1 (s) [b]	*19.1 (3.8)*	*19.3 (4.3)*	*20.1 (4.7)*	*19.3 (3.6)*	*19.3 (4.6)*	*18.8 (3.9)*	21.1 (5.8)	20.6 (5.4)	19.9 (4.7)	20.2 (3.5)	20.4 (4.3)	21.4 (4.2)
SCWT-A task 2 (s) [b]	*25.4 (4.2)*	*25.1 (5.1)*	*25.0 (4.8)*	*25.3 4.9)*	*25.6 (5.6)*	*26.1 (5.3)*	26.5 (5.7)	26.9 (6.1)	25.4 (5.3)	26.0 (5.1)	24.7 (4.8)	25.4 (4.1)
SCWT-A task 3 (s) [b]	*60.5 (20.0)*	*65.4 (25.7)*	*57.9 (14.0)*	*59.0 (18.8)*	*62.1 (30.9)*	*58.4 (22.1)*	63.2 (22.1)	64.7 (24.4)	60.7 (25.3)	65.6 (20.0)	71.2 (33.4)	65.2 (24.8)
DSST (symbols) [a]	*35.5 (10.7)*	*36.4 (10.4)*	*35.5 (10.0)*	*38.6 (10.0)*	*38.8 (9.5)*	*38.4 (11.3)*	34.7 (9.7)	35.0 (9.9)	35.5 (9.6)	35.5 (10.5)	35.2 (12.6)	34.4 (8.6)
VFT (words) [a]	*30.4 (9.9)*	*31.7 (8.9)*	*32.6 (8.0)*	*30.3 (11.0)*	*33.5 (12.3)*	*34.7 (14.2)*	29.7 (9.2)	30.6 (8.2)	36.1 (11.3)	27.7 (8.6)	31.4 (9.3)	33.8 (11.0)

WP= Walking Program; PAP= Placebo Activity Program; AVLT= Auditory Verbal Learning Test; SCWT-A= Stroop Colour Word Test- Abridged; s= seconds; DSST= Digit Symbol Substitution Test; VFT= Verbal Fluency Test; [a] Higher score indicates better performance; [b] Lower score indicates better performance; [c] Median (25^{th}; 75^{th} percentiles)

Appendix Table 2 Cognitive test performance at baseline and after six and 12 months in the FA/B12/B6-group and the placebo-group (mean (SD), unless indicated otherwise)

	Men and FA/B12/B6			***Men and placebo***			**Women and FA/B12/B6**			**Women and placebo**		
Outcome (unit)	*T0 (n=44)*	*T6 (n=44)*	*T12 (n=43)*	*T0 (n=41)*	*T6 (n=41)*	*T12 (n=38)*	T0 (n=34)	T6 (n=34)	T12 (n=28)	T0 (n=33)	T6 (n=33)	T12 (n=29)
MMSE [a, c]	*28 (28; 29)*	*28 (27; 29)*	*28 (27; 30)*	*29 (28; 29)*	*28 (28; 29)*	*29 (28; 29)*	29 (28; 30)	29 (28; 30)	29 (27; 30)	29 (28; 30)	28 (27; 29)	29 (27; 30)
AVLT 1-5 (words) [a]	*31.1 (7.5)*	*31.3 (9.8)*	*29.1 (8.9)*	*31.7 (7.2)*	*35.8 (9.4)*	*31.5 (9.3)*	34.9 (8.9)	35.1 (10.8)	33.7 (10.0)	34.1 (7.4)	33.7 (8.8)	31.2 (6.6)
AVLT 6 (words) [a]	*5.6 (2.7)*	*5.9 (3.2)*	*4.9 (2.9)*	*5.5 (2.1)*	*6.6 (2.8)*	*5.2 (2.5)*	6.4 (2.4)	6.8 (3.6)	5.7 (3.0)	6.0 (2.5)	5.9 (3.1)	5.4 (2.8)
SCWT-A task 1 (s) [b]	*19.6 (3.7)*	*19.7 (4.3)*	*19.5 (4.5)*	*18.9 (3.7)*	*18.9 (4.6)*	*19.2 (4.1)*	20.1 (5.3)	19.8 (5.2)	20.2 (4.3)	21.4 (4.6)	21.3 (4.6)	20.8 (4.8)
SCWT-A task 2 (s) [b]	*25.8 (4.1)*	*26.1 (4.4)*	*25.8 (4.4)*	*24.9 (5.1)*	*24.7 (6.3)*	*25.4 (5.8)*	25.3 (6.2)	25.0 (5.7)	25.0 (5.2)	27.0 (4.6)	27.0 (5.6)	25.6 (4.6)
SCWT-A task 3 (s) [b]	*62.6 (21.1)*	*65.5 (29.9)*	*61.0 (22.4)*	*56.4 (16.6)*	*61.4 (27.5)*	*54.8 (12.8)*	60.2 (18.1)	62.6 (25.0)	60.7 (26.3)	68.4 (23.5)	72.2 (30.1)	64.1 (24.1)
DSST (symbols) [a]	*36.2 (11.7)*	*35.7 (10.2)*	*36.2 (12.1)*	*38.3 (8.7)*	*39.9 (9.2)*	*38.1 (9.1)*	36.4 (10.9)	37.7 (11.6)	36.6 (10.3)	33.7 (8.9)	32.5 (10.0)	33.5 (7.7)
VFT (words) [a]	*28.3 (10.7)*	*31.2 (11.2)*	*31.8 (10.3)*	*32.5 (9.9)*	*34.3 (10.6)*	*36.0 (13.3)*	28.9 (9.2)	31.2 (8.2)	33.8 (10.1)	29.0 (8.9)	30.6 (9.2)	36.7 (12.1)

FA/B12/B6= Folic Acid, vitamin B12 and B6; AVLT= Auditory Verbal Learning Test; SCWT-A= Stroop Colour Word Test-Abridged; s= seconds; DSST= Digit Symbol Substitution Test; VFT= Verbal Fluency Test; [a] Higher score indicates better performance; [b] Lower score indicates better performance; [c] Median (25^{th}; 75^{th} percentiles)

DISCUSSION

Neither the walking program nor FA/B12/B6 supplementation improved cognition in these community-dwelling older adults with MCI within one year. The walking program, however, was efficacious in improving memory in men and memory and attention in women, in those with better attendance.

To our knowledge, this is the first intervention study with people with MCI from the general population. While this is a novel approach,[38;39]any benefits of the WP and FA/B6/B12-supplementation may have been overshadowed, because of the fluctuating course of MCI in community-based samples.[40] Longer interventions and follow-up may have provided better insight into the potential preventive effects of these interventions on cognitive decline. Unfortunately, this was not possible in the present study.

The exact mechanisms by which aerobic exercise and vitamin supplementation may affect cognition are not yet known and some overlap between the hypothesized mechanisms may exist in terms of 'vascular changes'. In theory, the factorial design of this trial allowed to examine possible interaction-effects between walking and vitamin supplementation. Unfortunately, due to logistical and financial constraints, it was not possible to recruit a larger sample size in order to have sufficient power for the examination of potential interaction. Therefore, the effect of both interventions was examined independently and results for one intervention were adjusted for the possible influence of the other.

The lack of effect may have also been caused by insufficient power due to the unanticipated post-hoc stratification for gender. Because gender was an effect modifier, reporting the results for both genders together would make no sense. This resulted in a decrease of statistical power to detect differences between FA/B12/B6-supplementation and placebo pills, and the walking program and the placebo activity program.

Vitamin supplementation

A benefit of FA/B12/B6 supplementation was observed only on attention in women. This is in line with the conclusions of recent meta-analyses, that published trials do not yet provide adequate evidence for an effect of supplementation with folic acid, vitamins B12 and B6, alone or in combination, on tests for cognitive function in people with normal or impaired cognitive function.[17-19;41] A possible explanation for the lack of effect in our study may be the absence of screening for baseline vitamin deficiencies or hyperhomocysteinemia. However, there was no main intervention effect on cognition in post-hoc analyses of participants with baseline folate deficiency and/or hyperhomocysteinemia (n=85, data not shown). A higher dose of vitamin B supplementation also appears to be unwarranted, since the FA/B12/B6 supplementation significantly improved homocysteine and vitamin B concentrations within six months (*The effects of exercise and folic acid, vitamins B12 and B6 on homocysteine concentrations in adults with mild cognitive impairment: Submitted)* These changes did not result in improved cognitive function. A longer duration of vitamin B supplementation may however be necessary for the consolidation of neurological and vascular changes in order to promote cognitive function. In contrast to short-term interventions, Durga et al. (2007) recently observed a beneficial effect on cognitive performance after three years of supplementation with 800 micrograms of folic acid in subjects with raised homocysteine and normal vitamin B12 concentrations at baseline.[42]

Walking Program

The lack of a main effect of exercise may have been caused by the moderate adherence to the exercise programs. It is important to mention that the correlation between general cognition and adherence was only small and non-significant. Moreover, MMSE scores at baseline and after six and twelve months did not differ significantly between adherent (≥ 75 % of the sessions) and non-adherent (< 75% of the sessions) participants (data not shown). While many previous studies have included data only from participants who completed the program, a strength of the present study is that data were analyzed according to the intention-to-treat principle, including all randomized participants with available data, irrespective of exercise adherence. As a result, data from many participants (30 out of 152) that did not attend a single exercise session were included in the intention-to-treat analysis. The inclusion of data from non-adherent participants may have underestimated the actual intervention effect in our study. The importance of adherence is underlined by the fact that the effect of the walking program on some of the cognitive outcomes increased with increasing adherence.

In our study, baseline physical activity levels were relatively high. Around 80 percent of the women and 90 percent of the men reported being at least moderately active for 30 minutes or more per day. In comparison, in the general Dutch population 65 to 75 years of age, only 60 percent of women and 70 percent of men make this claim.[43] Despite the high self-reported baseline activity levels, the WP was efficacious in improving aerobic fitness compared with the PAP *(Feasibility and Effectiveness of Two Group-Based Exercise Programs for Community-Dwelling Older Adults with Mild Cognitive Impairment. Submitted).* Since larger effects of exercise on cognition are expected in sedentary subjects,[5] it is possible that a threshold physical activity level exists, above which additional moderate-intensity physical activity does not result in further cognitive benefit.

It is also possible that the contrast between the programs was not large enough to induce between group differences. However, according to heart rate recordings, there were clear differences between the moderate intensity of the WP and the low intensity of the PAP. *(Feasibility and Effectiveness of Two Group-Based Exercise Programs for Community-Dwelling Older Adults with Mild Cognitive Impairment. Submitted).* Indeed, the use of a low-intensity placebo activity program with non-aerobic exercise in the present study could be seen as a strength, as it offered the opportunity to blind both participants and instructors to the exercise intervention, and to exclude the Hawthorne effect of attention.

Although the rigorous design of our study (including the intention-to-treat analysis, blinding of participants, exercise instructors and outcome assessors, and a range of valid measurements to assess cognition) reduces the likelihood of bias, it may also explain the absence of beneficial main effects. Etnier et al. (1997)[44] performed a thorough meta-analysis on the effect of exercise on cognition, and found that the effect size decreased as experimental rigor increased. It must be noted that this meta-analysis was published in 1997 and did not include more recent methodologically sound positive trials.

Unfortunately, our results do not correspond with two recent meta-analyses on the effects of exercise on cognitive function in cognitively healthy and demented subjects, which found significant positive effects.[5;6] The conclusions of these meta-analyses may have shifted to a more

positive view as a result of publication bias.[45] Publication bias may arise from the tendency of journals to reject studies without a positive outcome, and from the fact that authors are less likely to submit statistically non-significant results for publication.[46] Dissemination of both 'positive' and 'negative' findings is of great importance for science development as it provides better insight into which interventions are effective and which are not.

In conclusion, our results do not support the role of vitamin B supplementation in improving cognitive function in people with MCI. However, our findings suggest that regular participation in moderate-intensity walking may improve aspects of cognition, especially memory, in community-dwelling people with MCI. Regular participation in moderate-intensity physical activity should therefore be encouraged in this population group. Future larger trials are needed to confirm these findings. Another direction for future research is to examine ways to increase exercise frequency among this population.

What is already known on this topic:
- Cognitive impairment is a public health concern, since it occurs frequently among older people and their number is growing
- Aerobic exercise and vitamin B supplementation may benefit cognitive function
- The effectiveness of these interventions has not been examined yet in community-dwelling people with mild cognitive impairment.

What this study adds:
Neither a moderate-intensity walking program, nor high dose vitamin B supplementation was effective in improving cognitive function of community-dwelling older adults with mild cognitive impairment within one year. The walking program, however, was efficacious in improving memory in men and memory and attention in women with better adherence.

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