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Effects of multivitamin, mineral and herbal supplement on cognition in younger adults and the contribution of B group vitamins

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Objective Cognitive benefits of multivitamins have been observed in the elderly, but fewer trials have investigated younger, healthy cohorts. This randomised, double-blind, placebo-controlled study investigated the cognitive effects of 16-week multivitamin supplementation in adults aged 20–49 years.
Method A total of 138 participants aged 20–50 years were randomised and 116 completed the trial. The participants completed a computerised battery of cognitive tasks before and after 16-week supplementation with a multivitamin containing minerals and herbs or placebo. Blood measures of homocysteine, vitamin B6, B12 and folate were collected at both time points.
Results In men, there was a strong trend ($p = 0.01$; which did not reach significance when adjusted for multiple comparisons) for the multivitamin to improve performance on the incongruent stroop task, a measure of selective attention and response inhibition. There were no cognitive benefits of multivitamin supplements in women. Multivitamin supplementation substantially increased blood levels of vitamin B6, B12 and folate in both genders and decreased homocysteine in men. In men who received the multivitamin, improved stroop congruent performance was associated with increased vitamin B6 levels.
Conclusion Multivitamin supplementation may be useful for maintaining levels of B vitamins. The effects of multivitamins on speeded attention such as the stroop task in young adults warrant further investigation.

KEY WORDS—multivitamin; cognition; memory; attention; B vitamins; homocysteine

INTRODUCTION

Multivitamin use is becoming increasingly popular amongst the general public and although the greatest proportion of regular multivitamin users are the elderly, usage amongst younger adults is also growing (Rock, 2007). The increasing popularity of these supplements has led to interest in the influence of multivitamins on a variety of health and behavioural outcomes. To date, randomised controlled trials were conducted to investigate the cognitive effects of multivitamins in children (Benton, 2012) or the elderly (Kennedy and Haskell, 2011), with fewer studies focussing on a mature adult demographic.

The extent to which multivitamin supplements are capable of improving cognition remains uncertain. It is likely that heterogeneous participant characteristics, multivitamin formulations and cognitive endpoints have contributed to this lack of clarity. Although several trials have demonstrated no impact of multivitamins on mental performance (Cockle *et al*., 2000; Wolters *et al*., 2005), a recent meta-analysis of 10 trials has indicated that there are some benefits to immediate recall following a minimum of 1 month's multivitamin supplementation (Grima *et al*., 2012). As only two studies included in this meta-analysis focussed exclusively on pre-midlife samples (Haskell *et al*., 2010; Kennedy *et al*., 2010), it may not be appropriate to generalise these findings to younger adults.

Adding to the findings of Grima *et al*. (2012), there is some evidence that multivitamin supplementation can yield improvements to memory in older adults (Harris *et al*., 2012; Macpherson *et al*., 2012). Notably, 8-week supplementation exerted benefits to computerised measures of episodic memory in middle aged to elderly men (Harris *et al*., 2012) and 16-week supplementation improved the speed of working memory in elderly women (Macpherson *et al*., 2012). Both episodic and working memories are cognitive faculties which are vulnerable to age-related decline

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(Ronnlund *et al*., 2005). These findings indicate that in older adults any benefits of multivitamin supplementation may be restricted to cognitive processes which are already operating at a sub-optimal level.

The question is then raised as to whether there would be any cognitive enhancing effects of multivitamins in young, healthy adults who are free from any cognitive deterioration. In a 9-week trial investigating women aged 25–50 years, multivitamin-related improvements were observed on a computerised multitasking framework consisting of memory search, mathematical, stroop and numerical processing tasks (Haskell *et al*., 2010). Similarly, cognitive performance has been modulated on a highly demanding serial subtraction task following 33-day multivitamin supplementation in men of a slightly older (35–55 years) age range (Kennedy *et al*., 2010). These findings suggest there may be some improvements to fluid intelligence-based attention processes in younger adults, but this premise requires further investigation.

Consequently, the present study investigated the cognitive effects of 4-month multivitamin, mineral and herbal supplementation in adults aged 20–50 years. Cognition was assessed using a computerised cognitive battery, which has demonstrated sensitivity to nutraceutical intervention in older adults (Pipingas *et al*., 2008; Harris *et al*., 2012). Given the younger cohort in the present study, it was hypothesised that similar to Haskell *et al*. (2010), computerised measures of processing speed and attention would be most responsive to supplementation with the multivitamin.

If multivitamins are capable of enhancing mental function, then it is important to elucidate the mechanism by which this occurs. In addition to vitamins and minerals, the supplements used in this study contain grape seed, green tea extract and ginkgo biloba. These plant extracts contain flavonoids, which possess purported cognitive enhancing effects (Spencer, 2008); however, the doses included in these supplements are small to sub-therapeutic. Alternately, B vitamins are known to influence mental function. Deficiency in B vitamins has been linked to increased levels of homocysteine, which may have a detrimental effect on cognition and brain structure towards the later life span (Firbank *et al*., 2010). In contrast to these epidemiological findings, randomised controlled trial results regarding influence of individual or combined B vitamins on cognition have been inconsistent (Deijen *et al*., 1992; Bryan *et al*., 2002). In the current study, we examined changes in blood levels of vitamin B6, B12, red blood cell (RBC) folate and homocysteine as potential mechanisms of cognitive enhancement. Previous studies using the same (Harris *et al*., 2012), or similar (Macpherson *et al*., 2012) multivitamins containing minerals and herbs have indicated that these supplements are capable of increasing levels of B vitamins and reducing homocysteine. In addition to the application of increased circulating levels of the B vitamins and folate and decreased levels of homocysteine as biomarkers of compliance and bioavailability, we hypothesised that the magnitude of these changes would be associated with changes in the cognitive performance.

EXPERIMENTAL METHODS

Trial design

The trial was a 16-week, randomised, placebo-controlled, double-blind, parallel group investigation. Participants were tested at baseline after 8-week and 16-week supplementations. Approval for the study was obtained from the Swinburne University Human Research Ethics Committee. Written informed consent was obtained from all participants. The trial was registered with the Australian New Zealand Clinical Trial Registry (ANZCTR number 12611000092998).

Participants

Participants were recruited from the community between February and August 2011 by way of newspaper adverts, flyers, radio, television and social media. The participants were healthy, non-smoking adults, aged 20–50 years, who were currently in at least part-time employment or studying. A wide age range was used to ensure that the participants were not solely a convenience-based sample of university students. Exclusion criteria included current or history of anxiety disorders, depression, psychiatric disorders, epilepsy, heart disease, high blood pressure, diabetes, alcohol abuse, head injury or stroke. Individuals were ineligible to participate if they had health conditions that would affect food metabolism including gluten/wheat allergies, kidney disease, liver disease, irritable bowel syndrome, coeliac disease or peptic ulcers. The participants were required not to take medications, herbal extracts, vitamin supplements or illicit drugs, which can influence cognition or mood for four weeks prior to (and the duration of) the study. The participants were requested to refrain from taking any form of medication within 5 days of admission (except for contraceptive pill, prophylactic antibiotics or other routine medications to treat benign conditions, such as antibiotics to treat acne). In total, 30 men and 38 women were randomly allocated to receive the multivitamin containing minerals and

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herbs treatment, and 30 men and 40 women were allocated to receive placebo.

Treatment

Male participants received the Swisse Men's Ultivite Multivitamin Mineral and Antioxidant with Herbs Formula 1® (Swisse Vitamins Pty Ltd., Melbourne, Australia), and female participants received the corresponding Swisse Women's formula. The Swisse multivitamin major ingredients include B vitamins as well as vitamins C, D and E, together with metal chelates and small quantities of selected botanicals. A complete list is shown in Table 1. The placebo tablets were matched in size and colour to the multivitamin tablets and included a small amount of riboflavin (2 mg) so as to provide a similar urine colouration effect. The participants were instructed to supplement with one tablet with breakfast daily for the 16 weeks. To prevent potentially acute supplementation effects, on days with scheduled study appointments, the participants were required to take the tablet after the study visit.

Randomisation and blinding

Randomisation was conducted by the supplier (Swisse Vitamins Pty Ltd., Melbourne, Australia) using a computer-generated random sampling set. Randomisation was achieved using a permuted block design with block size of 6. Men and women were randomised separately. The tablets were dispensed in identical blister packs labelled with the day of the week. Placebo and multivitamin treatments were packaged in identical opaque boxes, which were numbered according to the randomisation schedule. The blinding list was held by an investigator not involved in the data collection.

Calculation of sample size

Prior studies have indicated that the cognitive effects of multivitamins are in the range of small to moderate effects (Grima *et al*., 2012; Macpherson *et al*., 2012). For this trial, power analysis was conducted using G*Power 3.1.2. For a repeated measures design with two groups (treatment vs placebo) and three time points (baseline, 8 weeks and 16 weeks), it was determined that there would be an 80% chance of discovering a medium effect size difference ($f = 0.25$) between treatment groups with a total sample size of 86 participants (alpha level = 0.05). Considering that different treatments were required for men and women, it was determined that a sample size of 80 women and 80 men would be sufficient for detecting the main effects of medium effect size and interactions between treatment and time points.

Table 1. Ingredients of the multivitamin formulations

Ingredient	Women	Men	Ingredient	Women	Men
Beta-carotene	5 mg	5 mg	Parsley (*Petroselinum crispum* herb)	10 mg	10 mg
Vitamin D3	200 IU	200 IU	Tyrosine	—	1 mg
Vitamin E	50 IU	50 IU	Extracts equivalent to dry		
Vitamin B1	50 mg	30 mg	Fennel (*Foeniculum vulgare* fruit)	15 mg	15 mg
Vitamin B2 (Riboflavin)	50 mg	30 mg	Horsetail (*Equisetum arvense* herb)	30 mg	30 mg
Nicotinamide	50 mg	30 mg	Celery(*Apium graveolens* seed)	20 mg	20 mg
Vitamin B5 (Pantothenic acid)	68.7 mg	64.13 mg	Ginger (*Zingiber officinale* root)	15 mg	5 mg
Vitamin B6	41.14 mg	24.68 mg	Astragalus (*Astragalus membranaceus* root)	50 mg	50 mg
Vitamin B12 (Cyanocobalamin)	50 mcg	30 mcg	Gotu kola (*Centella asiatica* herb)	10 mg	50 mg
Biotin	50 mcg	50 mcg	Hawthorn (*Crataegus monogyna* fruit)	30 mg	100 mg
Folic acid	500 mcg	500 mcg	Chamomile (*Matricaria recutita* flower)	15 mg	—
Vitamin C (Ascorbic acid)	165.2 mg	165.2 mg	Licorice (*Glycyrrhiza glabra* root and stolon)	10 mg	—
Choline bitartrate	25 mg	25 mg	Bearberry (*Arctostaphylos uva-ursi* leaf)	25 mg	—
Inositol	25 mg	25 mg	Barberry (*Berberis vulgaris* root)	—	15 mg
Lysine hydrochloride	50 mg	50 mg	Siberian ginseng (*Eleutherococcus senticosus* root)	25 mg	—
Citrus bioflavonoids extract	40 mg	40 mg	Sarsaparilla (*Smilax officinalis* root)	—	50 mg
Calcium	42 mg	21 mg	Buchu (*Barosma betulina* leaf)	—	10 mg
Magnesium	47.16 mg	57.89 mg	Damiana (*Turnera diffusa* leaf),	—	120 mg
Potassium	2 mg	4 mg	Extracts equivalent to fresh		
Iron	4.9 mg	3 mg	Oats (*Avena sativa* herb)	500 mg	500 mg
Chromium	6.2 mcg	6.2 mcg	Globe artichoke (*Cynara scolymus* leaf)	50 mg	50 mg
Manganese	1.6 mg	1.2 mg	Standardised extracts equivalent to		
Copper	58 mcg	28 mcg	Grape seed (*Vitis vinifera* seed dry)	1 g	1 g
Iodine	50.46 mcg	50 mcg	Green tea (*Camellia sinensis* leaf dry)	20 mg	20 mg
Zinc	5 mg	6 mg	Ginkgo (Ginkgo biloba leaf dry)	5 mg	100 mg
Selenium	26 mcg	26 mcg	Bilberry (*Vaccinium myrtillus* fruit fresh)	25 mg	25 mg
Co-enzyme Q10 (Ubidecarenone)	1 mg	1 mg	St. Mary's thistle (*Silybum marianum* fruit dry)	50 mg	50 mg
Spearmint oil	1.5 mg	1.5 mg	Tomato (*Lycopersicon esculentum* fruit)	700 mg	700 mg
Papaya (*Carica* papaya fruit powder)	10 mg	10 mg	Korean ginseng (*Panax* ginseng root dry)	—	50 mg
Lutein	200 mcg	200 mcg	Saw palmetto (*Serenoa repens* seed dry)	—	200 mg

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Cognitive measures: pre-treatment and post-treatment

The Swinburne University Computerised Cognitive Assessment Battery (SUCCAB) is a validated, reliable, computerised test battery of tasks designed to capture the range of cognitive functions that decline with age (Pipingas *et al*., 2010). SUCCAB tasks have previously demonstrated sensitivity to the cognitive enhancing effects of multivitamin supplementation in middle aged (Harris *et al*., 2012) and elderly individuals (Macpherson *et al*., 2012). The SUCCAB tasks were presented via computer, and responses were recorded using a hand-held button box. The tests used in this study have demonstrated good construct validity and test–retest reliability; full details have been published previously (Pipingas *et al*., 2010).

Outcomes were the response time on the following eight SUCCAB tasks:

Attention and processing speed

Simple reaction time. Response to a single white square, presented at random intervals.

Choice reaction time. Choice response to red squares and blue triangles presented at random intervals.

Stroop—congruent and incongruent. Response to the words RED, YELLOW, GREEN and BLUE presented in colours either congruent or incongruent with the word.

Memory

Immediate and delayed recognition memory. Immediate recognition after viewing a series of abstract images and delayed recognition at the end of the test battery (20-min delay).

Contextual recognition memory. Pictures of everyday items were presented in the top, bottom, left or right of the screen. The participants responded to the original location of the pictures when they were presented again in the centre of the screen.

Working memory. The participants remembered the location of the six white squares in a 4×4 grid.

Arrow flankers

Arrow flankers are computerised tests of attention in the presence of distracters. Five symbols appear on screen, with a centre arrow pointing to the left or right. The participants press the right or left key corresponding to the direction of a central arrow, which is surrounded by pairs of squares, congruent arrows (pointing in the same direction) or incongruent arrows (pointing in the opposite direction). The outcome for this task is the response time.

Blood measures

The participants fasted from 10 PM the previous night and were scheduled for fasting blood samples to be collected between 8.30 AM and 9.30 AM on the following morning. A blood sample was collected via venipuncture. Samples were sent by courier to a commercial pathology laboratory for analysis. Duplicate sample analysis was not conducted. The following samples were analysed.

Homocysteine and vitamin B12. About 8.5 ml of blood was collected in a serum separator tube containing clot activator (silicone and micronized silica). The blood was left to clot at room temperature before being centrifuged. A competitive immunoassay using direct, chemiluminescent technology was used to analyse serum samples. The ADVIA Centaur homocysteine kit has an assay precision of less than 7% total coefficient variation, and the ADVIA Centaur VB12 kit has an assay precision of less than 11% total coefficient variation.

Vitamin B6. About 4 ml of blood was collected in a tube containing anticoagulant (heparin) for the analysis of vitamin B6 levels, this tube was wrapped in foil to prevent degradation of the sample by light. Levels of vitamin B6 were determined from whole blood using high-performance liquid chromatography (HPLC) direct analysis of pyridoxal-5-phosphate form. The Chromsystems reagent kit for the HPLC analysis of B6 has an inter-assay precision of $<4\%$ coefficient variation.

RBC folate. About 4 ml of blood was collected in a tube containing anticoagulant (ethylenediaminetetraacetic acid) for the analysis of RBC folate levels. This tube was wrapped in foil to prevent degradation of the sample by light. A competitive immunoassay using direct, chemiluminescent technology was used to analyse serum samples of RBC folate. The ADVIA Centaur Folate kit has an assay precision of less than 10% total coefficient variation.

Procedure

The participants attended four sessions at Swinburne University. The participants underwent screening and practised all cognitive tasks during the first (practise) session. The participants were required to fast from 10 PM the night before the baseline appointment. During baseline testing, a fasting blood sample was collected between 8:30 AM and 9:30 AM, via venipuncture.

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The participants were then provided with a standardised breakfast of wholemeal toast or cereal with skim milk. The participants completed the computerised cognitive test battery, which took approximately 30 min. At each session, the participants reported their dietary intake of fruit and vegetables (0–4 serves daily). A number of questionnaires related to mood and general health were also completed (Pipingas *et al*., 2013). Supplementation commenced the day after the baseline visit. Post-treatment testing was held 8 weeks and 16 weeks later and followed the same protocol as baseline. Alternate versions of cognitive tests were used at each testing session.

Statistical analysis

The primary outcome was the response time for the correct responses on the SUCCAB measures and arrow flankers task. Only response times were analysed as ceiling effects that have been identified for numerous SUCCAB accuracy measures in older adults (Harris *et al*., 2012; Macpherson *et al*., 2012), and it was anticipated that due to the younger sample in this study, ceiling effects may be apparent on multiple measures. Only the baseline and 16-week data were included in these analyses to capture the cognitive effects over the full term of the trial. One-way analysis of covariance (ANCOVA) was used to examine the effect of treatment (multivitamin and placebo) on response time at 16-week post-treatment, with the baseline scores included as the covariate. This was conducted for each cognitive measure. Analyses were carried out separately for men and women due to differences in the multivitamin formulations. For the individual cognitive measures, the significance level of 0.05 was Bonferroni-adjusted for multiple tests of the same cognitive domain: a correction of 0.05/4 ($p < 0.013$) was applied to the four memory measures and 0.05/5 ($p < 0.01$) for the five attention measures (including arrow flankers). On the identification of a significant treatment group effect, or a trend for this effect, *t*-tests were used to examine the difference between baseline and post-treatment measures for each treatment group individually. The same ANCOVA procedure was adopted for the analysis of biochemical results. The significance level of 0.05 was Bonferroni-corrected for the three measures of B vitamins at a level of 0.05/3 ($p < 0.017$).

To determine whether baseline or changes in biochemical levels influenced cognitive changes, ANCOVA was conducted for cognitive measures that showed a significant effect of treatment or a trend ($p < 0.1$) for such an effect. Specifically ANCOVA was used to examine the effect of treatment on change in the cognitive score, with the baseline cognitive scores, baseline blood levels, change in blood levels and treatment x change in blood levels included as covariates. Separate ANCOVAs were conducted for vitamins B6, B12, RBC folate and homocysteine.

RESULTS

Demographics

In total, 56 of the participants that were assigned the multivitamin and 60 who were allocated the placebo completed the trial. Nine participants from the multivitamin group withdrew their consent to participate, one participant was lost to follow-up and two withdrew because of increased feelings of fatigue, potentially attributed to the treatment. Six participants from the placebo group were lost to follow-up and four withdrew their consent. The age range of participants was 20–49 years. Demographics for participants who completed the trial are shown in Table 2. Independent samples *t*-tests indicated that at baseline, there were no significant differences between the multivitamin and placebo groups in terms of age or years of education (including primary, secondary and tertiary education; 13 years indicate completion of primary and secondary school). About 66% of the sample was currently studying tertiary education. A breakdown of the number of hours per week spent in employment and study is published elsewhere (Pipingas *et al*., 2013).

Baseline gender differences

In terms of demographics, independent samples *t*-tests revealed there were no age differences between gender; however women reported slightly higher education than men ($t(114) = -2.16$, $p = 0.03$). In terms of biochemical measures, blood levels of RBC folate were higher in women than men ($t(108) = -5.14$, $p < 0.001$), and homocysteine levels were lower in women ($t(111) = 2.27$, $p = 0.03$). There were no significant gender differences in baseline levels of vitamins B6 or B12.

Daily fruit and vegetable intake

The participants were asked to report about their usual pattern of food consumption over the last 12 months. The majority of the participants (68%) reported

Table 2. Means, standard deviations and statistical significance values from independent t tests comparing demographic variables for the multivitamin and placebo groups

	Gender	*n*	Multivitamin	*n*	Placebo	*p*
Age	Male	24	28.9 (6.9)	28	30.9 (7.5)	0.32
	Female	32	32.8 (7.7)	32	31.2 (6.8)	0.39
Years of education	Male	24	16.3 (1.9)	28	17.0 (2.6)	0.33
	Female	32	17.7 (3.0)	32	17.9 (3.0)	0.84

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consuming one serving of fruit daily at baseline. A comparable intake was reported at follow-up. Pearson's Chi-square statistics indicated that fruit intake did not differ according to the treatment group or gender at either testing session. Most participants reported that they consumed two servings of vegetables daily (36%), and 30% of participants consumed one serving of vegetables daily. Intake was comparable at follow-up. Pearson's Chi-square statistics indicated that vegetable intake did not differ according to the treatment group. Gender differences in vegetable intake were apparent at baseline only (Pearson's $\chi^2(3,\ n=116)=13.84,\ p=0.003$), with women reporting higher intake.

Cognitive measures

The results of the cognitive analyses are summarised in Table 3. At both time points, performance was at, or close to, ceiling (100% accuracy) for the majority of the participants for simple reaction time, stroop congruent and incongruent, spatial working memory and arrow flankers. Average accuracy ranged from 70% to 85% for the other memory measures.

Means, standard deviations, *F* and statistical significance values from the ANCOVA analysis to determine treatment effects are shown in Table 3. For the memory measures, there were no significant effects of treatment. For the attention measures, an effect of treatment was identified for men for the stroop congruent measure ($F(1,49)=6.43,\ p=0.01,\ \eta^2=0.12$) but only represented a trend once accounting for multiple comparisons. Paired *t*-tests revealed that there was a non-significant reduction in stroop congruent response time for the multivitamin group and a significant increase in response time for the placebo group ($t\ (27)=-3.03,\ p=0.005$). A trend for treatment was also identified for men for the stroop incongruent measure ($F(1,46)=3.78,\ p=0.06,\ \eta^2=0.08$). There were no significant effects of treatment for the other attention measures, including arrow flankers.

Biochemical markers

Means, standard deviations, *F* and statistical significance values from the ANCOVA analysis are shown in Table 4. Treatment effects were significant for all blood measures with the exception of homocysteine in women. For the multivitamin treatment, *t*-tests revealed that there was a significant increase in vitamin B12 in women ($t\ (29)=-7.58,\ p=0.000$)

Table 3. Means, standard deviations, change form baseline, *F* and statistical significance values from the ANCOVA analysis for response time (ms) at baseline and post-treatment on SUCCAB and arrow flankers cognitive tasks for the multivitamin and placebo groups

		Multivitamin				Placebo					
Cognitive task	Gender	*n*	Baseline	Post-treatment	Δ	*n*	Baseline	Post-treatment	Δ	*F*	*p*
Simple reaction RT	Male	21	293 (25)	293 (35)	0	28	275 (27)	279 (28)	+4	0.51	0.48
	Female	30	287 (41)	282 (35)	−5	31	295 (43)	290 (30)	−5	0.44	0.51
	Total	51	289 (34)	287 (34)	−2	59	286 (37)	285 (30)	−1	0.02	0.90
Complex reaction RT	Male	20	411 (39)	425 (67)	+14	22	394 (48)	399 (50)	+5	0.68	0.41
	Female	27	416 (58)	416 (70)	0	25	404 (46)	427 (62)	+23	1.18	0.28
	Total	47	414 (50)	419 (68)	+5	47	399 (47)	414 (58)	+15	0.11	0.75
Stroop congruent RT	Male	24	643 (110)	622 (106)	−21	28	617(90)	648 (109)	+31	6.43	0.01*
	Female	31	642 (123)	644 (122)	+2	31	645 (102)	650 (113)	+5	0.0.4	0.84
	Total	55	643 (117)	634 (115)	−9	59	632 (97)	649 (110)	+17	2.93	0.09
Stroop incongruent RT	Male	24	757(121)	735 (110)	−22	25	748 (131)	775 (113)	+27	3.78	0.06
	Female	30	721 (125)	732 (108)	+11	31	739 (109)	732 (108)	−7	0.06	0.81
	Total	54	737 (123)	733 (108)	−4	56	743 (118)	755 (108)	+12	1.56	0.22
Contextual recognition RT	Male	24	803 (85)	795 (106)	−8	25	835 (104)	854 (139)	+19	1.45	0.24
	Female	29	817 (101)	818 (117)	+1	29	819 (121)	786 (120)	−33	2.59	0.11
	Total	53	811 (93)	808 (111)	−3	54	827 (113)	818 (132)	−9	0.01	0.91
Immediate recognition RT	Male	21	902 (93)	867 (78)	−35	28	969 (101)	933 (91)	−36	3.00	0.09
	Female	29	907 (113)	876 (130)	−31	28	892 (84)	890 (101)	−2	1.94	0.17
	Total	50	904 (104)	871 (110)	−33	56	930 (99)	912 (98)	−18	2.19	0.14
Delayed recogniton RT	Male	22	942 (84)	936 (82)	−6	22	978 (101)	936 (81)	−42	0.80	0.38
	Female	28	933 (115)	916 (97)	−17	30	914 (111)	923 (109)	+9	0.55	0.46
	Total	50	937 (102)	925 (90)	−12	58	945 (110)	948 (118)	+3	1.18	0.28
Spatial working memory RT	Male	22	765 (134)	700 (145)	−65	27	798 (125)	751 (147)	−47	0.83	0.37
	Female	30	867 (160)	784 (151)	−83	30	802 (149)	753 (146)	−49	1.04	0.31
	Total	52	824 (156)	748 (143)	−76	57	800 (137)	752 (145)	−48	2.72	0.10
Arrow flankers	Male	23	489 (54)	476 (70)	−13	26	507 (60)	496 (58)	−11	0.14	0.71
	Female	28	530 (64)	504 (63)	−26	28	507 (70)	482 (53)	−25	0.41	0.52
	Total	51	511 (62)	491 (67)	−20	54	507 (64)	489 (55)	−18	0.00	0.96

RT, response time (msecs).
*$p < 0.05$.

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and men ($t(20) = -4.35$, $p = 0.000$), RBC folate in women ($t(28) = -5.38$, $p <= 0.001$) and men ($t(19) = -5.17$, $p = 0.000$), vitamin B6 in women ($t(29) = -11.21$, $p < 0.001$) and men ($t(20) = -9.61$, $p = 0.000$) and reduction in homocysteine in men ($t(20) = 4.02$, $p = 0.001$). For the placebo, there was an increase in RBC folate in both men ($t(25) = -2.91$, $p = 0.007$) and women ($t(28) = -4.22$, $p < 0.001$), whereas all other B vitamin biochemical measures were non-significant, see Table 4.

Relationship between biomarkers and cognitive change

Analysis of covariance analyses were conducted in men to determine whether the observed treatment-related cognitive changes to stroop congruent response time were related to baseline biochemical blood levels or changes in biochemical levels over the 16-week supplementation period. The treatment effect was not significant with the addition of the covariates RBC folate, homocysteine and vitamin B12, and change in stroop performance was not related to blood levels at baseline, change in blood levels or blood level change × treatment interaction for any of these biochemical measures. However, for vitamin B6, change in stroop performance was significantly related to vitamin B6 change × treatment interaction ($F(1,42) = 8.45$, $p = 0.006$). Changes in levels of vitamin B6 versus predicted cognitive changes from the ANCOVA model in men are plotted in Figure 1. Pearson's correlations demonstrated a moderate negative correlation for the multivitamin (Pearson's $r = -0.51$, $p = 0.017$) indicating that reduced stroop response time was related to the increasing levels of vitamin B6, whereas there was a trend for the opposite in the placebo group (Pearson's $r = 0.38$, $p = 0.052$).

DISCUSSION

Findings from this study indicate that 4-month supplementation with multivitamins containing minerals and herbs did not yield consistent cognitive benefits in healthy adults aged 20–49 years. Although there was

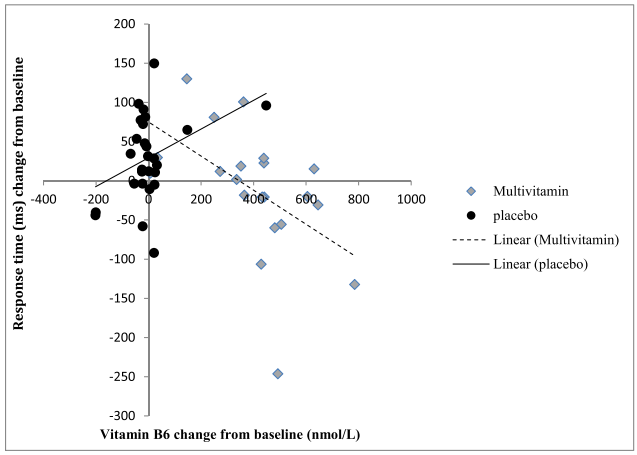

Figure 1. Scatter plot displaying change in vitamin B6 levels from baseline to post-treatment and predicted change in stroop congruent response time from the ANCOVA model in men. Positive values for vitamin B6 change indicate increased vitamin B6 levels from baseline to post-treatment, whereas negative values for stroop congruent performance indicate improved (faster) response times. A moderate negative correlation is shown for the multivitamin, indicating that reduced response time was related to increasing levels of vitamin B6, whereas there is a trend for the opposite in the placebo group

Table 4. Means, standard deviations, *F* and statistical significance values from the ANCOVA analysis for blood vitamin B and homocysteine levels at baseline and post-treatment for the multivitamin and placebo groups

		Multivitamin				Placebo					
Biochemical measure	Gender	*n*	Baseline	Post-treatment	Δ (%)	*n*	Baseline	Post-treatment	Δ (%)	*F*	*p*
Vitamin B12 (pmol/l)	Male	21	323 (127)	418 (106)	+29	27	333 (102)	323 (99)	−3	25.63	0.000***
	Female	29	275 (87)	379 (113)	+38	30	307 (102)	331 (121)	+8	12.09	0.001*
	Total	57	296 (107)	395 (111)	+33	57	319 (102)	327 (110)	+3	32.87	0.000***
RBC folate (nmol/l)	Male	20	862 (233)	1048 (240)	+22	26	835 (119)	916 (151)	+10	6.76	0.013*
	Female	29	1029 (207)	1392 (256)	+35	29	1024 (127)	1143 (215)	+12	15.91	0.000***
	Total	49	961 (231)	1252 (300)	+30	55	935 (155)	1036 (218)	+11	19.64	0.000***
Homocysteine (μmol/l)	Male	21	11.3 (2.3)	9.9 (1.7)	−12	28	11.3 (2.3)	11.45 (2.2)	+1	11.81	0.000***
	Female	30	10.6 (2.2)	8.7 (1.7)	−18	30	10.3 (1.6)	9.4 (2.0)	−9	3.67	0.060
	Total	51	10.9 (1.6)	9.2 (1.8)	−16	58	10.7 (1.6)	9.2 (1.8)	−14	15.92	0.000***
Vitamin B6 (nmol/l)	Male	21	119.5 (66)	513 (215)	+362	27	126 (83)	124 (140)	−2	87.03	0.000***
	Female	30	114 (73)	584 (228)	+412	29	108 (67)	137 (187)	+27	74.04	0.000***
	Total	51	113 (60)	555 (224)	+391	56	117 (75)	131 (165)	+12	156.6	0.000***

RBC, red blood cell.
*$p < 0.05$.
***$p < 0.001$.

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a trend for the treatment effect on the stroop measure in men, this was not attributed to a significant reduction in the response time for the multivitamin group. The multivitamin did not influence performance on any other cognitive tasks. Multivitamin supplementation resulted in increased levels of RBC folate, vitamin B6 and B12 in both genders, with men also demonstrating a significant reduction in homocysteine. Furthermore, in men who received the multivitamin, decreased stroop congruent response time across the study period was associated with increased vitamin B6 levels.

The results provide limited support for the prediction that multivitamin supplementation would preferentially improve measures of processing speed and attention. Because of the application of stringent Bonferroni corrections to the cognitive measures, there was a trend and not a statistically significant effect for multivitamin supplementation to improve response time on the congruent stroop task in men. A similar, yet non-significant, pattern of results was also identified for the incongruent stroop task. Processing speed has been demonstrated to account for a large proportion of the variance in both the stroop congruent and incongruent measures (Pipingas *et al*., 2010). However, as there were no benefits of multivitamin supplementation to the arrow flankers, simple and choice reaction time measures, it is unlikely that the improvements on the stroop task were solely because of alterations in processing speed. The incongruent stroop task also indexes selective attention, inhibition and executive function (Pipingas *et al*., 2010), and similar processes underlie performance of flankers tasks (Heil *et al*., 2000). Haskell *et al*. (2010) have identified multivitamin-related improvements to the incongruent stroop and mathematical calculation components of a multitasking framework in women of similar age to the current study, and 12-week multivitamin supplementation improved attention measures including the arrow flankers task in children (Haskell *et al*., 2008). In the current study, multivitamin supplementation did not benefit these abilities in women, and effects in men were inconsistent.

Multivitamin supplementation did not enhance memory performance. A previous trial conducted in older adults has demonstrated that the same duration of multivitamin supplementation improved speed of spatial working memory response (Macpherson *et al*., 2012). In middle aged men, the same male multivitamin formula used in the current study improved episodic memory (Harris *et al*., 2012). Episodic and working memories decline with age (Ronnlund *et al*., 2005) and there may be less scope to improve these processes in healthy young adults with optimum cognitive function. Indeed, in the current study, working memory accuracy reached a ceiling effect; therefore, there was a limited scope for benefits to this measure. In addition, baseline biochemical measures in the current study indicated that the sample was within normal reference ranges for vitamins B6, B12 and folate. In general, the elderly are at higher risk of B vitamin nutritional deficiency than younger adults (Joosten *et al*., 1993), and it may be that cognitive benefits of nutritional interventions will be larger in those with inadequate vitamin intake or deficiency.

Despite adequate levels of B vitamins at baseline, 4-month multivitamin supplementation further increased B vitamin blood levels. Homocysteine significantly decreased in men who received the multivitamin and women in both treatment groups. Although larger increases in RBC folate were observed for both genders for the multivitamin, an increase in RBC folate was observed for the placebo. Dietary folate is available in fresh leafy vegetables, asparagus, broccoli, mushrooms and legumes (Braun and Cohen, 2009). As baseline testing occurred in winter, and post-treatment in spring and summer, seasonal changes in food nutrient content may have contributed to alterations in RBC folate and homocysteine in the placebo group.

The current study demonstrated that multivitamin supplementation decreased homocysteine by 12% in men, and these reductions were comparable with older men (Harris *et al*., 2012) and slightly smaller than the observations in women of a similar age range (Haskell *et al*., 2010). The multivitamin increased the levels of RBC folate by approximately 30% in both genders, slightly larger than the previous findings in an older group of men using the same vitamin formulation (Harris *et al*., 2012). Increases of vitamin B12 by around 33% were comparable with older men (Harris *et al*., 2012) and women (Macpherson *et al*., 2012). Substantially, larger increases (3–4-fold) were observed for vitamin B6, particularly in women. There is growing evidence that cardiovascular risk factors, such as elevated homocysteine may need to be addressed at midlife, or earlier, to prevent later occurring vascular damage to the brain (Vuorinen *et al*., 2011). Thus, reducing homocysteine in younger years and the maintenance of low levels of homocysteine may hold potential to prevent vascular and neural pathologies later in the life span.

In the current study, alterations to levels of homocysteine were not associated with changes to cognitive performance. Instead, the results indicated that in men, increased levels of vitamin B6, due to multivitamin supplementation, were associated with

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the improvement to stroop congruent performance. In a cross-sectional investigation of healthy young through to elderly individuals, greater vitamin B6 blood levels have been related to better performance across multiple cognitive domains, ranging from visual–spatial organisation to working memory, scanning–tracking, and abstract reasoning (Elias *et al*., 2006). In a sample of women, 35-day vitamin B6 supplementation demonstrated small benefits to verbal fluency but not to other cognitive measures (Bryan *et al*., 2002), and in elderly men, 3-month vitamin B6 treatment improved long-term memory but not short-term memory (Deijen *et al*., 1992). Our finding that the vitamin B6 in the multivitamin may contribute to cognitive enhancements requires further investigation.

The reason why small benefits to processing speed were restricted to men is unclear, especially as cognitive benefits were associated with increases in vitamin B6, and the female supplement contained larger quantities of vitamins B6 and B12. Differential gender effects may be due to variances in the multivitamin formulas or diverging dietary requirements. The quantities of antioxidants such as beta-carotene, vitamin C and vitamin E did not differ between the two formulas; however, 100 mg of ginkgo biloba was found in the male supplement, and only a sub-therapeutic dose of 5 mg was included in the female formula. Ginkgo biloba has demonstrated acute attentional effects in young adults (Elsabagh *et al*., 2005) and therefore may have contributed to the small attentional changes in men in this study. In regard to gender differences at baseline, women reported greater consumption of vegetables than men, and women also had higher blood levels of RBC folate and lower homocysteine. The lack of cognitive effects in women may be due to better health status at baseline.

More important, this study focussed on the chronic effects of multivitamins at 16-week end point given that the participants did not take a multivitamin on the day of return testing. The results therefore represent effects due to the long-term accumulation of multivitamin constituents in the body. As previous studies have shown that multivitamins may also provide acute health benefits in the hours following ingestion (Kennedy *et al*., 2008), this is an area for future investigation given the potential for additive chronic and acute effects. Future studies should also investigate more targeted age ranges to determine whether there are certain stages prior to midlife, which demonstrate the greatest benefits from multivitamin supplementation. In conclusion, this study of healthy adults, multivitamin supplementation yielded minimal improvements to attention measures and these findings were only apparent in men. The identification of a relationship between increasing levels of vitamin B6 and improved attention speed in men requires further investigation.

CONFLICT OF INTEREST

The National Institute of Integrative Medicine, of which Professor Avni Sali is currently the director, receives financial support from Swisse Vitamins Pty Ltd. Avni Sali and Andrew Pipingas are on the Scientific Advisory Board for Swisse Vitamins Pty Ltd. Aside from the oversight of the study design, Swisse Vitamins Pty Ltd and Avni Sali were not involved in any other aspects of the conduct of the trial including analysis or interpretation of the trial findings.

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