ORIGINAL INVESTIGATION

Effects of high-dose B vitamin complex with vitamin C and minerals on subjective mood and performance in healthy males

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Abstract

Rationale A significant proportion of the general population report supplementing their diet with one or more vitamins or minerals, with common reasons for doing so being to combat stress and fatigue and to improve mental functioning. Few studies have assessed the relationship between supplementation with vitamins/minerals and psychological functioning in healthy cohorts of non-elderly adults.

Objectives The present randomised, placebo-controlled, double–blind, parallel groups trial assessed the cognitive and mood effects of a high-dose B-complex vitamin and mineral supplement (Berocca®) in 215 males aged 30 to 55 years, who were in full-time employment.

Methods Participants attended the laboratory prior to and on the last day of a 33-day treatment period where they completed the Profile of Mood States (POMS), Perceived Stress Scale (PSS) and General Health Questionnaire (GHQ-12). Cognitive performance and task-related modulation of mood/fatigue were assessed with the 60 min cognitive demand battery. On the final day, participants also completed the Stroop task for 40 min whilst engaged in inclined treadmill walking and subsequent executive function was assessed.

Results Vitamin/mineral supplementation led to significant improvements in ratings on the PSS, GHQ-12 and the 'vigour' subscale of the POMS. The vitamin/mineral group also performed better on the Serial 3s subtractions task and rated themselves as less 'mentally tired' both pre- and post-completion of the cognitive demand battery.

Conclusions Healthy members of the general population may benefit from augmented levels of vitamins/minerals via direct dietary supplementation. Specifically, supplementation led to improved ratings of stress, mental health and vigour and improved cognitive performance during intense mental processing.

Introduction

Optimum functioning of the central nervous system is dependent on a wide range of micronutrients. For example, folic acid and vitamins B12 and B6 play specific roles in the methylation and decarboxylation processes that govern the integrity of DNA, and the synthesis of DNA, proteins, phospholipids and monoamine and catecholamine neurotransmitters (Mattson and Shea 2003). All three vitamins also play key roles in the remethylation and metabolism of the potentially toxic amino acid homocysteine, increased levels of which may contribute to a range of neurodegenerative and psychiatric disorders via a host of deleterious effects to cellular, haemodynamic, oxidative and vascular parameters (Mattson and Shea 2003). Vitamin C is also the brain's most prevalent antioxidant and is found at its

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greatest concentrations in neuron rich areas. Evidence suggests that its one-electron donor properties make it an essential co-factor in a plethora of processes that underlie neural maturation, neuroprotection and neurotransmission (Harrison and May 2009). Similarly, a number of minerals, including zinc, magnesium and calcium, are essential for adequate functioning of the central nervous system, and play diverse roles including in myelination, neurotransmitter synthesis and modulation of activity in the dopaminergic and adenosinergic neurotransmitter systems (Black 1996; Cardoso et al. 2009; Gareri et al. 1995).

There is a wealth of evidence from epidemiological studies that clearly suggest a relationship between micronutrients and psychological functioning. The largest body of work, mainly conducted in older cohorts, has demonstrated that circulating levels of one or more B vitamins and/or homocysteine are related to depression (Bjelland et al. 2003; Kim et al. 2008; Sanchez-Villegas et al. 2009; Tiemeier et al. 2002), and cognitive performance (Durga et al. 2006; Duthie et al. 2002; Elias et al. 2006, 2008; Feng et al. 2006; Kado et al. 2005; Vidal et al. 2008). However, the inter-relationship between B vitamins and homocysteine levels and the causality of the relationships with brain function have not yet been adequately elucidated (Elias et al. 2006). A number of epidemiological studies conducted in older participants have also demonstrated a relationship between cognitive performance, or risk of dementia, and vitamin C intake (Del Parigi et al. 2006; Engelhart et al. 2002).

The case for direct intervention with supplements as assessed by controlled trials is less clear. Three recently updated Cochrane reviews examined the efficacy of vitamin B6 (Malouf and Grimley Evans 2003); B12 (Malouf and Sastre 2003) and B9 with or without B12 (Malouf and Grimley Evans 2008) in attenuating the cognitive declines associates with age and dementia. These reviews all concluded that there is no consistent evidence for efficacy on the basis of the small number of studies that met their inclusion criteria. The authors recommend that further work is needed in this area. A trial conducted by Durga et al. (2007), cited in the latter review as being methodologically adequate, showed that 3 years supplementation with folic acid in 818 50- to 70-year-old participants, selected on the basis of high homocysteine levels, led to decreased homocysteine and improved memory function, information processing and sensorimotor speed. The authors also note a number of methodological concerns across the studies in this area, including the use of insensitive instruments such as the Mini-Mental State Exam, which may account for the lack of consistent evidence in this area. As with B vitamins, the evidence for direct supplementation with vitamin C in elderly or dementia suffering cohorts is also somewhat equivocal (Morris 2009).

In terms of supplementation with a fuller range of vitamins and minerals, a number of studies have been conducted in cohorts of children. Benton (2001), reviewing the results of the effects of multi-vitamins/minerals on children's intelligence (IQ) across 13 studies, noted some evidence of improved performance that was restricted to non-verbal tests of intelligence. Following a subsequent meta-analysis, Eilander et al. (2010) concluded, on the basis of a non-significant trend, that there was a 'possibility of a small positive effect' of supplementation on similar measures of fluid intelligence and evidence of a significant improvement in academic achievement from a small sub-set of studies. A further randomised, placebo-controlled study has also been completed after the inclusion period for these reviews. Haskell et al. (2008) demonstrated benefits to cognitive performance in children during 12 weeks supplementation with a multi-vitamins/mineral. With regards adults, Benton et al. (1995a, b) showed that 12 months supplementation with a high-dose multi-vitamin was able to improve subjective mood (Profile of Mood States—POMS), mental health (General Health Questionnaire—GHQ) and cognitive performance on attention tasks. However, these effects were restricted to the females within the sample (Benton et al. 1995a, b). Schlebusch et al. (2000), in a placebo-controlled trial involving 300 healthy adults, found that 4 weeks administration of a vitamin B complex with vitamin C and minerals (Berocca Calmag®) improved subjective ratings of stress, anxiety and psychological well-being. Similarly, Carroll et al. (2000), assessing the effects of a similar supplement (Berocca®) in a sample of 80 healthy males, also reported significantly attenuated subjective stress (Perceived Stress Scale—PSS) and anxiety (Hospital Anxiety and Depression Scale) in healthy males following 4 weeks supplementation.

The current randomised, double-blind, placebo-controlled, parallel groups trial therefore assessed the effects of 33-days supplementation with a high-dose B vitamin complex plus vitamin C and minerals (Berocca®) on cognitive performance, mood and well-being in healthy males in full-time employment. High levels of cognitive demand, and the resultant mental fatigue during testing, may provide a sensitive backdrop for the measurement of treatment-related cognitive enhancement. Laboratory-induced mental fatigue has also been shown to disrupt tasks that require high levels of executive control of cognitive processes (Kato et al. 2009; Lorist et al. 2009), and these fatigue-related decrements may also afford a more sensitive backdrop for assessing enhancement. This exploratory study therefore employed a cognitive demand battery (CDB) that elicits mental fatigue and which has been shown to be sensitive to a number of natural interventions (Kennedy et al. 2008; Reay et al. 2005) and included the performance of executive function tasks during

a state of fatigue inculcated by extended cognitive task performance and physical activity.

Materials and methods

Participants

Participants were recruited via email distribution, local press adverts, publicity on local radio and posters in the Newcastle upon Tyne area. Following screening and informed consent ($N=244$) a total of 215 healthy males aged 30–55 years were randomised and completed this exploratory trial. During the blind data review process, further five participants were removed for major protocol violations (one non-compliance to treatment, one significant medical condition, three body mass index (BMI)>40), giving a per protocol sample of 210 evaluable participants (multi-vitamin/mineral, $N=103$; placebo, $N=107$). Demographic data for each group are given in Table 1.

Screening comprised training and familiarisation with tasks as well as ensuring participants met the inclusion criteria of being male, aged 30–55 years and in full-time employment and did not breech any exclusion criteria. These included having a clinically significant medical condition within the last 5 years, use of medication within the previous 5 days, use of any over-the-counter vitamins and/or other dietary supplements within the previous 4 weeks or any relevant food intolerances/sensitivity. Information relating to demographics and baseline characteristics of the two groups was also obtained at this point. This included age, BMI and average consumption of alcohol, cigarettes and fruit and vegetables. Consumption levels were measured simply by self-report following advice regarding what, according to government guidelines, constituted a portion in the case of fruit and vegetables and a unit in the case of alcohol.

Treatments

Participants received either placebo or a multi-vitamin/mineral supplement (Berocca®) in identical effervescent tablets. Participants ingested one tablet per day with water for the 33 (±2) days of the study. The multi-vitamin/mineral tablets contained vitamins: B1 (15 mg), B2 (15 mg), B6 (10 mg), B12 (10 mcg), C (500 mg), biotin (150 mcg), folic acid (400 mcg), nicotinamide (50 mg) and pantothenic acid (23 mg) and minerals: calcium (100 mg), magnesium (100 mg) and zinc (10 mg). Participants were assigned to treatment groups in accordance with a computer-generated randomisation schedule provided by the manufacturer and were dispensed the study product designated to the corresponding randomisation number. Participants returned all unused treatment at the final visit. Compliance (between 80% and 120% of the treatment) was assessed via tablet counting and treatment diary cards.

Cognitive and mood measures

With the exception of the paper and pencil questionnaires all tasks and visual analogue scales were administered using the Computerised Mental Performance Assessment System (COMPASS—Kennedy and Haskell under review), a platform for the presentation of computerised tasks derived from standard psychometric measures.

Fatigue and mood

GQH-12 (Goldberg et al. 1997) The General Health Questionnaire is a screening instrument used for assessing

Table 1 Demographics for the placebo and multi-vitamin/mineral groups

	Placebo ($N=107$)		Multi-vitamin/mineral ($N=103$)	
	Mean	SD	Mean	SD
Age	38.8	6.35	39.7	7.08
Height	1.79	0.07	1.80	0.64
Weight	85.6	12.5	86.6	12.0
BMI	26.6	3.37	26.8	3.38
Systolic blood pressure	136	13.5	136	13.7
Diastolic blood pressure	88.0	9.66	87.6	9.51
Heart rate	71.0	11.4	70.1	12.1
Respiratory rate	16.6	4.89	16.8	6.29
Temperature	35.9	1.00	35.9	0.87
Fruit and vegetables (portions per day)	3.20	1.48	3.71	2.00
Alcohol (units per week)	15.0	10.9	13.3	11.0
Cigarettes (no. per day)	1.41	4.77	0.88	2.75

general psychological health both in clinical settings and in non-clinical research settings requiring repeated measurements over time. The GHQ-12 consists of 12 items, each assessing the severity of a mental problem over the past few weeks using a 4-point scale (from 0 to 3) with higher scores indicating worse conditions (Goldberg and Williams 1988).

PSS (Cohen et al. 1983) The PSS is a 10-item questionnaire that assesses the degree to which situations in one's life are appraised as stressful using a 5-point scale (0–4). It is a widely used research instrument and its validity has been established within a number of populations (Froelicher et al. 2004; Golden-Kreutz et al. 2004; Mimura and Griffiths 2004).

POMS (McNair et al. 1992) The POMS is a well-established, factor-analytically derived measure of psychological distress for which high levels of reliability and validity have been documented. The POMS consists of 65 adjectives rated on a 0–4 scale that can be consolidated into 'depression-dejection', 'tension-anxiety', 'anger-hostility', 'confusion-bewilderment', 'vigour-activity' and 'fatigue-inertia' sub-scales. The latter two sub-scales can be interpreted as measures of fatigue and have been validated as separate factors in a number of studies. Norms have been published for a variety of patient and non-patient groups.

Bond–Lader mood scales (Bond and Lader 1974) The Bond and Lader scales have been utilised in numerous pharmacological, psychopharmacological and medical trials. These scales comprise a total of 16 100 mm lines anchored at either end by antonyms (e.g., 'alert-drowsy', 'calm-excited'). Participants indicate their current subjective position between the antonyms on the line. Outcomes comprise three factor analysis derived scores: 'Alertness', 'Calmness' and 'Contentment'.

Energy visual analogue scales Visual analogue scales (VAS) anchored at either end by 'very low' and 'very high' assessed the participants' self-rated levels of 'concentration', 'mental stamina' and 'physical stamina'. VAS anchored at either end by 'not at all' and 'extremely' assessed the participants' self-rated fatigue levels of being 'mentally tired' and 'physically tired'.

Cognitive tasks

CDB This battery is designed to assess the impact of treatment on speed/accuracy and mental fatigue during continuous performance of cognitively demanding tasks. The working hypothesis underlying this approach is that any psychoactive properties of a test substance are liable to be more readily apparent during a period of intense cognitive demand and the 'mental fatigue' state elicited by this prolonged task performance.

In the current study, the battery performed a second role as part of the mental component in the inculcation of mental and physical fatigue in participants. Application of this battery in its usual 60 min form has been shown to reliably increase self-ratings of 'mental fatigue' and to be sensitive to a number of natural interventions (Kennedy et al. 2008; Kennedy and Scholey 2004; Reay et al. 2005). The effects of this battery tend to increase towards the later stages of completion. The 10-min battery (which is repeated six times) comprises:

Serial subtractions (3s and 7s—2 min each)
The original verbal Serial 7s test has appeared in a number of forms, including as part of the Mini-Mental State Examination for dementia screening. In the current study modified, 2-min computerised versions of the Serial 3s and Serial 7s tests were utilised (Scholey et al. 2010). At the start of each 2-min section, a standard instruction screen informed the participant to count backwards in threes or sevens, as quickly and accurately as possible, using the linear number keys of the keyboard to type in each response. Participants were also instructed verbally at the outset that if they were to make a mistake they should carry on subtracting from the new incorrect number. A random starting number between 800 and 999 was presented on the computer screen, which was cleared by the entry of the first response. Each three-digit response was represented on screen by an asterisk. Pressing the enter key signalled the end of each response and cleared the three asterisks from the screen. Performance data (number of correct subtractions and number of errors performed during the 2 min task) were calculated for the Serial 3s and 7s elements, separately. In the case of incorrect responses, subsequent responses were scored as positive if they were correct in relation to the new number.

Rapid visual information processing task (5 min)
In this task, the participant monitored a continuous series of digits for targets of three consecutive odd or three consecutive even digits. The digits were presented at the rate of 100 min^{-1} and the participant responded to the detection of a target string by pressing a response key as quickly as possible. The task was continuous and lasted for 5 min, with eight correct target strings being presented in each minute. The task was scored for percentage of target strings correctly detected, average reaction time for correct detections and number of false alarms.

Subjective 'mental fatigue' scale
Following each repetition of the battery of tasks, participants rated their subjective feeling of 'mental fatigue' via an on-screen 100-mm visual analogue scale with the

end-points labelled 'not at all' and 'extremely'. The scales were scored as percent along the line towards 'extremely'.

Stroop task

Whilst performing 40 min of inclined treadmill walking (Reebok 3-series treadmill), titrated to keep the participants heart rate at approximately 60% of the maximum heart rate for their age, participants performed the Stroop task. This task was used to both inculcate further mental fatigue and measure cognitive performance. This was a computerised version of a commonly used classic task (Stroop 1935), which requires attentional and central executive resources. In this case the task ran for 40 min, with the data collapsed into four 10-min epochs. Words describing one of four colours ('RED', 'YELLOW', 'GREEN', 'BLUE') were presented in different coloured fonts in the centre of a computer screen stationed at eye level in front of the treadmill. The participant pressed one of four coloured response buttons mounted on the treadmill in order to identify the font colour (e.g., if the word 'GREEN' was presented in a blue font, the correct response would be to respond with the blue button). The presented words were either 'congruent' (word and font are the same colour) or 'incongruent' (word and font are different colours) and were presented in a random order. The task was scored for reaction times and accuracy of responses to 'congruent' and 'incongruent' words.

Executive functioning tasks

The following classic 'executive' tasks, which have been shown to be sensitive to fatigue (van der Linden et al. 2003), were utilised.

Peg-and-ball task

In this computerised version of the original 'Tower of London', executive function task participants were presented with two configurations of three coloured balls (blue, green, red) on three pegs that held three, two and one ball, respectively. The participants had to rearrange the balls from the starting configuration so that they matched the position of the balls in the goal configuration, moving one ball that did not have a ball above it at a time. Participants were asked to work out the entire planning sequence and calculate the minimum number of moves required prior to moving the balls using the mouse/cursor. Participants completed a total of 18 trials, comprising six trials each that could be solved in three, four and five moves, respectively, in ascending order of difficulty. Each trial generated scores for planning times prior to moving, time to complete and number of moves made above the minimum required to complete the task.

Wisconsin card sort task (Berg 1948)

This is a standard clinical measure that is commonly used as a measure of executive functioning and is thought to require cognitive flexibility, problem solving and the use of feedback to guide behaviour. In this computerised version of the test, participants were asked to match cards that varied by colour, shape and number to four 'key cards'. Participants were not told how to sort the cards, but were required to determine the correct category from the feedback given to their responses, which changed periodically throughout the test. In total the current version comprised the presentation of up to 128 cards. Overall accuracy, percentage of perseverative and non-perseverative errors committed and time to complete during the test were used as the measure of executive functioning.

Procedure

Within 14 days of the initial screening visit, participants arrived at the laboratory at 08.30 am (±30 min) for their pre-treatment assessment (day −2). They completed an assessment of mood and well-being comprising the GHQ-12, the PSS, the POMS and Bond–Lader mood scales. Immediately after this they completed the CDB for 60 min. Fatigue was assessed immediately before and after completion of the CDB with the 'Energy VAS'.

Participants were then dispensed their treatment for the 33 (±2)-day treatment period and instructed to take one tablet dissolved in a full glass of water (8 oz or 250 ml of water) every morning after waking beginning 2 days following the laboratory visit and thereafter throughout the study treatment period. They were also given a diary card to record intake of tablets and any general health observations.

Participants returned after 33 days of treatment (day 33± 2 days where necessary) and completed exactly the same assessments. In addition to those tasks completed at baseline (pre-treatment assessment), they also completed the Stroop task for 40 min whilst engaged in inclined treadmill walking. They then completed the 'Energy VAS' for a final time before executive function was assessed with peg-and-ball and Wisconsin card sort tasks. The timelines of the two laboratory visits are shown in Fig. 1. Details of the tasks and timelines for the final laboratory visit (day 33±2) are shown in Fig. 2.

Statistics

Prior to analysis of the day 33 data, any baseline differences on the performance and questionnaire measures and any underlying cohort differences in terms of demographic and lifestyle variables were assessed with one-way (treatment) ANOVA.

Fig. 1 Timelines for the pre-treatment (day −2) and post-treatment (day 33±2) visits to the laboratory
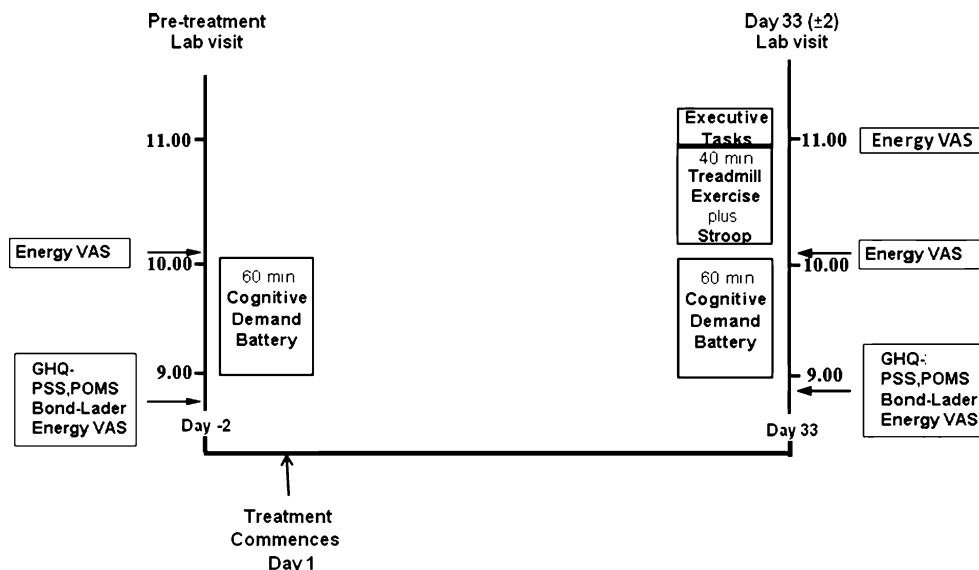
Questionnaires

All total and subscale scores for the GHQ-12, PSS, POMS and Bond–Lader questionnaires on day 33 were analysed with one-way (treatment) ANCOVA using the respective pre-treatment score as a covariate.

CDB scores, mental fatigue and subjective fatigue/energy during completion

Scores for the six repetitions of the CDB and ratings on the 'Mental Fatigue' and the two repetitions of the 'Energy VAS' (completed before and after the CDB) on day 33 were

Fig. 2 Testing schedule during the final laboratory visit (day 33±2 days)

analysed with two-way (treatment × repetition) ANCOVA using the respective averaged pre-treatment assessment score as a covariate.

Stroop task

Stroop scores were collapsed into four 10-minute epochs and analysed by two-way (treatment × epoch) ANOVA.

Executive function

The peg-and-ball task and Wisconsin card sort scores and the final 'Energy VAS' were analysed with one-way (treatment) ANOVA. For those measures that included more than one post-treatment assessment a priori planned comparisons were made using t tests calculated with the Mean Squares Error from the ANCOVA. In order to reduce the potential for Type I errors only those planned comparisons associated with a statistically significant difference or trend ($p < 0.1$) towards the same showing a treatment × repetition term are reported. Given this, and the exploratory nature of the study, the exponentially reducing probability of significant differences occurring for one treatment at two or more consecutive time points by chance, and an a priori statistical analysis plan for the study that precluded the report or interpretation of isolated significant differences, no correction was applied for family wise error rate across time points.

Power

A power calculation carried out a priori indicated that a total sample size of 200 would be adequate in order to have >80% power to detect differences between treatment groups of moderate effect size (in accordance with Cohen 1988).
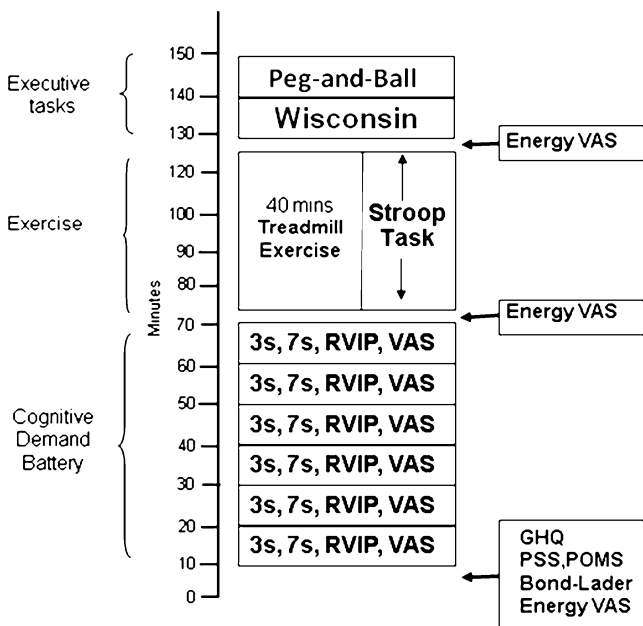
Results

Demographics and baseline measures

There were no significant baseline differences between the two groups on any performance/questionnaire measures. However, those in the multi-vitamins/minerals group reported consuming significantly more portions of fruit/vegetables on average per day than those in the placebo group [$F(1,208)=4.62$, $p<0.05$]. Therefore, in addition to pre-treatment score where applicable, fruit/vegetable intake was included as a further covariate in the analysis of post-treatment scores reported below. Results from ANCOVAs both with and without 'fruit and vegetable consumption' as a covariate are presented in Tables 2, 3 and 4.

Questionnaires

Treatment with multi-vitamins/minerals led to a number of significant improvements on the questionnaire meas-

ures. Participants ratings of their general mental health were improved in comparison to placebo, as evinced by reduced scores on the GHQ-12 [$F(1,205)=4.52$, $p<0.05$]. Similarly they reported lower subjective stress, as measured by the PSS, in comparison to placebo [$F(1,205)=5.24$, $p<0.05$].

Overall scores on the POMS also revealed a trend towards improved mood that narrowly failed to reach significance [$F(1,196)=3.77$, $p=0.054$] following multi-vitamins/minerals compared to placebo. Reference to the POMS sub-scales showed a significant increase in self-reported 'vigour' ratings [$F(1,196)=6.03$, $p<0.05$] and a non-significant trend [$F(1,196)=3.43$, $p=0.07$] towards reduced 'confusion' levels following treatment in comparison to placebo.

Unadjusted mean scores (plus SDs) and F scores from the ANCOVAS for the above measures are presented in Table 2. The adjusted means for those measures that showed significant effects, or trends towards the same, are presented graphically in Fig. 3.

Table 2 Pre- and post-treatment (day 33±2) questionnaire mood scores

Instrument	Subscale		N	Pre-treatment (day −2)		Post-treatment (day33)		ANCOVA[a]		ANCOVA[b] + fruit/veg covariate	
								F	p	F	p
GHQ		Placebo	107	9.86	3.37	9.14	3.70	3.63	0.06	4.50	<0.05
		Vitamin/mineral	102	9.99	3.62	8.30	3.61				
PSS		Placebo	107	13.0	5.25	12.9	5.54	4.07	<0.05	5.20	<0.05
		Vitamin/mineral	102	14.2	6.09	12.4	4.95				
POMS	Tension	Placebo	100	16.0	4.58	15.8	4.95	0.10	–	0.16	–
		Vitamin/mineral	100	17.2	5.90	16.3	5.23				
	Depression	Placebo	100	21.7	7.52	21.9	8.04	2.06	–	2.1	–
		Vitamin/mineral	100	22.4	8.45	21.1	7.12				
	Anger	Placebo	100	19.5	6.41	20.0	6.53	1.36	–	1.77	–
		Vitamin/mineral	100	20.3	7.47	19.6	6.35				
	Vigour	Placebo	100	24.7	5.63	25.2	5.60	6.18	<0.05	6.00	<0.05
		Vitamin/mineral	100	24.0	5.14	26.3	5.29				
	Fatigue	Placebo	100	14.5	5.01	14.3	6.12	1.62	–	2.10	–
		Vitamin/mineral	100	15.5	5.75	14.1	5.59				
	Confusion	Placebo	100	12.9	3.78	12.9	3.98	3.26	0.07	3.40	0.07
		Vitamin/mineral	100	13.8	4.13	12.7	3.97				
	Total Score	Placebo	100	60.2	26.6	59.7	28.0	3.37	0.07	3.77	0.05
		Vitamin/mineral	100	65.0	30.7	57.4	27.3				
Bond–Lader	Alert	Placebo	105	62.9	12.6	66.4	13.9	1.12	–	1.12	–
		Vitamin/mineral	98	61.4	12.6	67.1	14.6				
	Content	Placebo	105	66.0	13.1	68.6	14.3	0.92	–	0.92	–
		Vitamin/mineral	98	63.9	12.9	68.4	14.5				
	Calm	Placebo	105	62.2	12.5	65.7	14.4	0.30	–	0.30	–
		Vitamin/mineral	98	60.4	13.0	63.3	15.3				

Data are mean unadjusted scores plus standard deviations, with F scores and probabilities from a one-way ANCOVA of day 33 data using pre-treatment (day −2) scores as a covariate and with and without 'fruit/vegetable' consumption as an additional covariate

[a] ANCOVA including pre-treatment baseline data as a covariate

[b] ANCOVA including additional fruit/vegetable consumption covariate

CDB scores and changes in fatigue/energy
during completion

Performance of the CDB tasks was also improved in the
multi-vitamins/minerals group, with a significant main
effect of treatment [$F(1,199)=4.05$, $p<0.05$] showing that
participants made significantly more correct Serial 3s
subtractions in comparison to placebo across the six
repetitions of the tasks. There was also a trend towards a
treatment × repetition interaction on the number of correct
Serial 7s subtractions [$F(1,199)=1.93$, $p=0.09$]. Reference
to the planned comparisons revealed that participants made
significantly more correct responses following the first
repetition of the task [$t(995)=3.53$, $p<0.0005$], with a trend
towards the same during the second repetition [$t(995)=
1.73$, $p=0.08$]. There were no effects on the number of
errors. There were no interpretable effects on rapid visual
information processing.

In terms of the effects of task completion on ratings of
mental fatigue, there was a trend towards a beneficial
treatment related main effect on ratings of 'mental fatigue'
across repetitions of the CDB [$F(1,198)=2.90$, $p=0.09$] in
comparison to the placebo group. There was also a main
effect of treatment on the 'Energy VAS' ratings of 'mental
tiredness' made before and after the CDB [$F(1,198)=4.90$,
$p<0.05$] with those in the multi-vitamins/minerals group
reporting lower levels of 'mental tiredness' than those in
the placebo group across both ratings.

Unadjusted mean scores (plus SDs) from the CDB,
including F scores from the ANCOVAS, are presented in
Table 3 with data from the pre- and post-CDB completion
of the 'energy VAS' presented in Table 4. The adjusted
means for those measures that showed significant effects, or
trends towards the same, are presented graphically in Fig. 4.

Stroop and executive tasks

There were no significant effects of treatment on perfor-
mance of the Stroop, peg-and-ball or WCS tasks. Nor was
the final 'Energy VAS', completed following all tasks,
significantly modulated.

Discussion

The current study demonstrates that 33 days supplementa-
tion with a B complex, vitamin C and minerals product
(Berocca®) benefited healthy males in terms of significant-
ly improved ratings of general mental health (GHQ-12),
reduced subjective stress (PSS) and increased ratings of
'vigour' (POMS), with a strong trend towards an overall
improvement in mood as assessed by the POMS. Task
performance, in terms of the number of correct Serial 3s

subtractions throughout the six repetitions of the CDB
tasks, and Serial 7s during the first repetition, was also
improved. This was accompanied by reduced ratings of
'mental tiredness' during CDB task performance and a
trend towards reduced 'mental fatigue'. The analysis
omitting 'fruit and vegetable' consumption as a covariate
showed a similar pattern, albeit with slightly weaker results
on the GHQ-12 and POMS measures (see Tables 2, 3 and
4). Prior to treatment, there were no significant differences
between the placebo and multi-vitamin/minerals groups in
performance or ratings for any of the study outcomes.

The results presented here with regards the general
mental health and mood questionnaires directly confirm
previous findings in cohorts of healthy adults administered
the same or similar supplement. Specifically, Schlebusch et
al. (2000) found that 28 days supplementation led to
decreased anxiety, improved well-being and decreased
stress in both men and women and Carroll et al. (2000)
found that the same period of administration led, amongst
other effects, to lower GHQ scores and decreased ratings of
stress as assessed by the PSS in a sample of males. The
results are also broadly in line with those seen following
supplementation with multiple vitamins for a longer time
period (12 months) by Benton et al. (1995a), with the
exception that significant benefits to mood were seen only
in the females within their sample of 129 healthy adults.
However, it may be relevant that in this previous study only
vitamins were administered, but at very high doses, and the
sample employed was somewhat smaller than the current
study.

The benefits to task performance were largely restricted
here to an increase in correct subtractions across repetitions
on the Serial 3s task following vitamins/minerals, with a
similar effect seen only on the very first repetition of the
Serial 7s task. This differential treatment related enhance-
ment of the two tasks has been seen previously (e.g., Reay
et al. 2005; Scholey et al. 2009) and has been interpreted as
reflecting the differing cognitive demands of the two tasks.
In the case of the easier, and therefore faster, Serial 3s task,
successful completion requires resources in terms of
psychomotor function, attention, working memory and
modest amounts of executive function. However, the Serial
7s task is subjectively more difficult, has little reliance on
psychomotor speed (as response rates are approximately
halved), requires a similar amount of working memory but
entails the recruitment of much greater attentional and
executive resources. It is interesting to note that the
improvements in mental arithmetic tasks that require
attentional and executive resources are broadly in line with
improved performance seen in adult females on two
executive tasks (mental arithmetic and Stroop) within a
multi-tasking battery following 9 weeks supplementation
with multi-vitamins/minerals (Haskell et al. under review).

Table 3 Pre- and post-treatment (day 33±2) cognitive demand battery scores

Measure	Day		N	Repetition of task 1st	2nd	3rd	4th	5th	6th	Term	ANCOVA[a] F	p	ANCOVA[b] + fruit/veg covariate F	p
Serial 3s Correct (No)	D −2	Placebo	105	36.5 *12.5*	39.4 *13.0*	39.1 *12.7*	40.1 *13.3*	39.8 *12.6*	40.0 *13.2*	C	5.09	<0.05	4.05	<0.05
		Vitamin/mineral	98	34.7 *14.4*	37.7 *13.7*	37.8 *14.0*	37.1 *13.4*	38.2 *13.8*	38.4 *14.3*					
	D 33	Placebo	105	37.6 *12.9*	41.5 *13.7*	42.3 *13.2*	41.2 *13.2*	41.4 *12.9*	41.4 *13.6*	C × R	1.90	0.09	1.39	–
		Vitamin/mineral	98	37.7 *13.3*	40.5 *13.7*	40.2 *14.3*	41.2 *13.8*	41.3 *13.6*	41.7 *13.7*					
Serial 3s Errors (No)	D −2	Placebo	105	1.28 *1.46*	1.78 *1.87*	2.04 *2.04*	1.71 *1.81*	1.89 *1.71*	1.83 *1.89*	C	0.00	–	0.01	–
		Vitamin/mineral	98	1.62 *1.98*	2.05 *2.24*	1.98 *2.14*	2.20 *2.32*	2.07 *1.93*	1.97 *3.10*					
	D 33	Placebo	105	1.31 *1.64*	1.62 *1.80*	1.76 *1.63*	1.89 *1.78*	1.93 *2.06*	2.11 *1.99*	C × R	1.56	–	0.86	–
		Vitamin/mineral	98	1.36 *1.88*	1.89 *2.04*	2.46 *3.81*	2.11 *2.22*	1.83 *2.03*	1.82 *1.97*					
Serial 7s Correct (No)	D −2	Placebo	105	24.7 *11.0*	25.5 *10.8*	26.0 *11.7*	26.3 *11.5*	27.2 *11.5*	27.5 *11.0*	C	1.67	–	1.04	–
		Vitamin/mineral	98	23.7 *11.1*	25.0 *11.5*	24.7 *11.5*	25.3 *11.6*	26.2 *12.2*	26.5 *12.0*					
	D 33	Placebo	105	25.6 *10.8*	27.0 *10.9*	27.9 *11.5*	28.3 *11.3*	28.7 *10.8*	28.7 *12.2*	C × R	2.12	0.06	1.93	0.09
		Vitamin/mineral	98	26.5 *11.2*	27.2 *10.9*	27.5 *11.2*	27.7 *11.0*	27.7 *11.2*	28.1 *11.7*					
Serial 7s Errors (No)	D −2	Placebo	105	1.97 *1.91*	2.03 *1.90*	2.16 *1.89*	2.31 *2.25*	2.27 *2.27*	2.37 *2.15*	C	0.01	–	0.30	–
		Vitamin/mineral	98	2.22 *2.98*	2.36 *2.17*	2.36 *2.21*	2.59 *2.39*	2.57 *2.65*	2.45 *2.44*					
	D 33	Placebo	105	1.77 *1.73*	2.11 *1.68*	2.25 *1.78*	2.27 *1.96*	2.27 *2.06*	2.51 *2.01*	C × R	1.37	–	1.38	–
		Vitamin/mineral	98	1.56 *2.31*	2.17 *2.07*	2.09 *2.09*	2.60 *2.31*	2.81 *2.68*	2.74 *2.80*					
RVIP% Accuracy	D −2	Placebo	103	65.3 *19.4*	67.3 *16.9*	67.6 *17.7*	66.1 *19.6*	65.6 *18.6*	66.0 *17.7*	C	0.29	–	0.15	–
		Vitamin/mineral	99	66.1 *18.1*	66.7 *18.6*	67.2 *18.6*	66.2 *20.0*	67.1 *18.9*	67.1 *19.7*					
	D 33	Placebo	103	67.0 *18.7*	66.1 *17.7*	67.6 *19.3*	66.5 *20.0*	65.8 *18.2*	63.5 *20.3*	C × R	1.12	–	1.07	–
		Vitamin/mineral	99	68.2 *19.3*	69.0 *18.7*	68.6 *18.4*	67.3 *18.7*	64.9 *19.8*	64.6 *19.7*					
RVIP RT (msecs)	D −2	Placebo	103	465 *54.5*	467 *56.3*	457 *51.9*	459 *65.2*	452 *55.2*	443 *48.2*	C	0.01	–	0.25	–
		Vitamin/mineral	99	458 *55.7*	465 *62.6*	460 *61.1*	453 *53.6*	450 *57.7*	455 *61.4*					
	D 33	Placebo	103	457 *52.3*	458 *58.8*	453 *49.2*	443 *53.3*	449 *55.0*	447 *57.2*	C × R	0.71	–	0.39	–
		Vitamin/mineral	99	458 *56.9*	455 *57.6*	453 *54.0*	443 *54.8*	446 *50.4*	454 *54.5*					
RVIP FA (number)	D −2	Placebo	103	4.41 *9.17*	3.67 *5.22*	3.51 *5.00*	3.48 *5.21*	3.55 *4.85*	3.49 *5.21*	C	0.42	–	0.11	–
		Vitamin/mineral	99	4.53 *5.65*	4.98 *10.4*	4.07 *6.12*	4.14 *5.42*	3.82 *3.85*	3.88 *4.37*					
	D 33	Placebo	103	3.59 *4.98*	3.61 *4.25*	3.51 *4.70*	3.95 *6.67*	4.03 *5.47*	3.70 *4.82*	C × R	0.51	–	0.92	–
		Vitamin/mineral	99	3.38 *3.43*	3.66 *3.82*	3.36 *3.35*	3.80 *4.03*	4.15 *5.07*	4.19 *5.02*					
Mental Fatigue	D −2	Placebo	106	46.6 *17.6*	50.4 *19.1*	55.4 *20.6*	58.8 *21.7*	62.2 *22.6*	64.3 *21.8*	C	0.21	–	2.90	0.09
		Vitamin/mineral	101	49.3 *17.4*	53.5 *17.3*	59.2 *17.4*	64.2 *17.2*	66.6 *17.4*	69.2 *17.6*					
	D 33	Placebo	106	45.6 *19.1*	48.8 *20.8*	52.8 *21.8*	56.5 *22.6*	58.8 *23.1*	60.4 *23.2*	C × R	0.00	–	0.01	–
		Vitamin/mineral	101	44.0 *20.8*	47.9 *21.0*	52.4 *21.6*	56.2 *22.4*	58.4 *23.0*	60.1 *23.2*					

Data are mean unadjusted scores plus standard deviations, with F scores and probabilities from a two-way ANCOVA of day 33 data (treatment × repetition) using pre-treatment (day −2) scores as a covariate and with and without 'fruit/vegetable' consumption as an additional covariate

$D − 2$ pre-treatment (day −2), $D33$ post-treatment, C condition, R repetition

[a] ANCOVA including pre-treatment baseline data as a covariate

[b] ANCOVA including additional fruit/vegetable consumption covariate

Table 4 Pre- and post- (day 33±2) energy VAS ratings pre- and post-completion of the CDB

		N	Pre-treatment (day−2)				Post-treatment (day33)				Term	ANCOVA[a]		ANCOVA[b] + fruit/veg covariate	
			1st Assessment		2nd Assessment		1st Assessment		2nd Assessment						
Concentration	Placebo	105	61.9	15.0	47.6	20.7	62.8	16.3	48.3	20.7	Cond	1.29	–	1.40	–
	Vitamin/mineral	97	58.7	15.4	46.7	21.1	63.6	14.7	48.7	19.9	Cond × Ass	0.00	–	0.00	–
Mental Stamina	Placebo	105	60.4	15.2	42.8	17.6	61.5	16.2	44.4	21.0	Cond	1.16	–	1.28	–
	Vitamin/mineral	97	57.4	15.9	44.4	19.6	61.7	16.8	47.0	18.5	Cond × Ass	0.91	–	0.97	–
Physical Stamina	Placebo	105	63.0	16.4	55.6	18.8	65.2	17.4	56.8	18.9	Cond	0.00	–	0.01	–
	Vitamin/mineral	97	59.4	16.9	55.8	19.1	62.9	17.8	56.9	19.4	Cond × Ass	1.49	–	1.29	–
Mental Tiredness	Placebo	105	38.9	17.7	60.5	21.4	39.3	20.6	60.6	20.1	Cond	5.18	<0.05	4.90	<0.05
	Vitamin/mineral	97	42.1	20.3	62.4	18.6	35.0	20.7	58.8	20.7	Cond × Ass	0.60	–	0.59	–
Physical Tiredness	Placebo	105	36.7	19.7	44.1	19.8	34.1	19.0	41.7	20.9	Cond	0.34	–	0.46	–
	Vitamin/mineral	97	38.7	19.8	44.7	19.8	37.0	22.2	42.9	21.9	Cond × Ass	0.47	–	0.22	–

Data are mean unadjusted scores plus standard deviations, with F scores and probabilities from a two-way ANCOVA of day 33 data (treatment × repetition) using pre-treatment (day −2) scores as a covariate and with and without 'fruit/vegetable' consumption as an additional covariate

Cond condition, *Ass* assessment

[a] ANCOVA including pre-treatment baseline data as a covariate

[b] ANCOVA including additional fruit/vegetable consumption covariate

Overall, the underlying rationale for the choice of cognitive tasks employed in the study was that both the high cognitive demands of performing the difficult CDB battery tasks for 60 min and the general declines in executive task performance and increased mental fatigue that can be observed following extended cognitive task performance in the laboratory (Kato et al. 2009; Lorist et al. 2009) would provide a more sensitive paradigm for detecting treatment related effects. Whilst the improved performance on the Serial 3s task suggested that this may have been the case for the CDB portion of the assessment, there were no treatment related improvements seen during either the Stroop task or in performance of the two executive function tasks at the end of the assessment.

Recent Food Standards Agency surveys of the UK population show that small percentages of this and all other age/gender groups suffer biochemical deficiencies with regards each of the vitamins administered in the current study (Ruston et al. 2004), with a bias towards poorer micronutrient status in lower socio-economic status groups (Nelson et al. 2007). The sample employed here were healthy males in full-time employment. Evidence also suggests that those who participate in research tend to be better educated and more affluent than those who do not participate in research (Stopponi et al. 2009). Whilst no reference was made to any nutritional parameter as an inclusion factor and no direct analyte data was collected, the assumption was made here that the sample enjoyed typical nutritional status. However, the very fact of being able to improve mood, ratings of mental health and vigour and aspects of task performance by simple supplementation with B vitamins, Vitamin C and minerals indicates that the cohort must have been suffering from less than optimal micronutrient status at the outset. However, it is important to note that the pre-treatment scores and ratings on all of the measures employed in the study were equivalent between groups, suggesting that the results are not predicated on any pre-treatment differences in nutritional status between groups. Given that physiological levels of vitamin analytes were not established for practical reasons, the adequacy of the nutritional status of the cohort and whether the results seen here represent alleviation of deficiencies as currently defined or whether the current definitions of deficiency leave further room for functional improvement is something of a moot point. Overall, these results suggest that improving nutritional status, by supplementation if necessary, may be beneficial to males within the general population as a whole.

Interestingly, a recent study undertaken in a similar, unselected (on a nutritional basis) cohort of females who were administered a wider range of vitamins and minerals for 9 weeks demonstrated improved multi-tasking and reduced tiredness (Haskell et al. under review). In that study a sub-section of participants also volunteered to give blood samples and significant reductions in the blood levels of homocysteine were seen following supplementation in comparison to the placebo group. Whilst the relevance and causal effect of increased homocysteine levels with regards decrements in brain function have yet to be fully delineated (Elias et al. 2006), the changes seen in this potentially
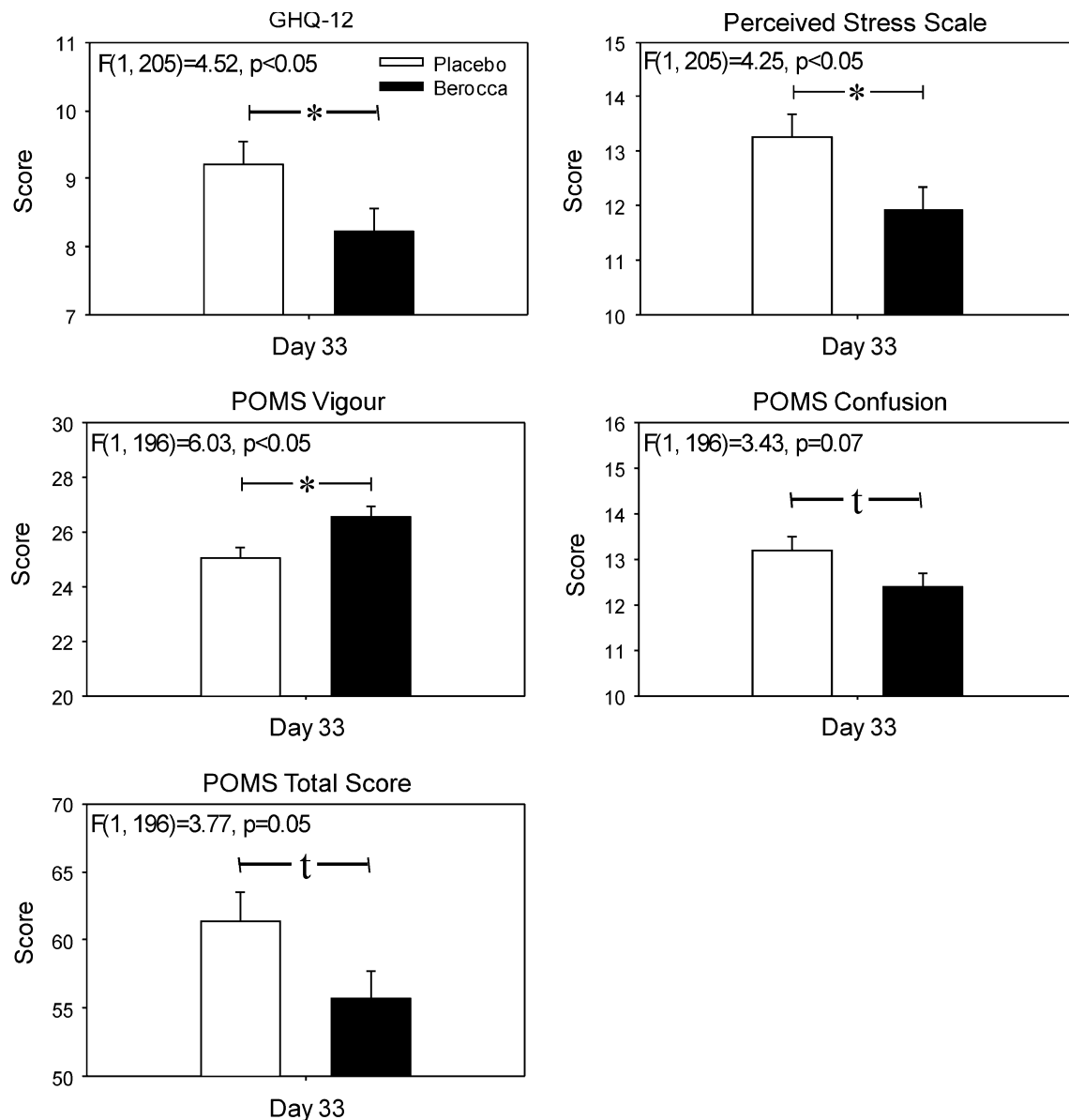
Fig. 3 Significant effects of multi-vitamins/minerals on ratings of general psychological health (*GHQ-12*), stress (*PSS*) and mood (*POMS*). Data shown are adjusted means from one-way ANCOVAS of day 33 data including pre-treatment scores and fruit/vegetable consumption as covariates. Reduced scores on the measures equate to benefits, with the exception of 'POMS vigour' (*$p<0.05$, t $p<0.1$)

important physiological parameter also suggest less than optimal micronutrient status in a similar cohort of females.

Naturally, given the multiple components of the supplement administered here it is not possible to identify the specific component(s) or physiological mechanism(s) underlying the beneficial effects. Whilst, a reduction in homocysteine is plausible, the B vitamins also contribute to the integrity and synthesis of monoamine and catecholamine neurotransmitters, DNA, proteins, and phospholipids via their roles in decarboxylation and methylation processes (Mattson and Shea 2003). Vitamin C is the brain's most prevalent antioxidant and is found at its greatest concentrations in neuron rich areas. Its one-electron donor

properties also make it an essential co-factor in a plethora of neural maturation, neuroprotection and neurotransmission processes relevant to brain function including as a modulator of cholinergic, GABAergic, dopaminergic and glutamatergic activity (Harrison and May 2009). Vitamin C was also included here at a dose (500 mg) that approximates to the intake necessary to fully saturate plasma and circulating cells in healthy adults (Levine et al. 2001) but which is far in excess of the recommended daily allowance of 60 mg (which is based on the levels necessary to prevent deficiency diseases).

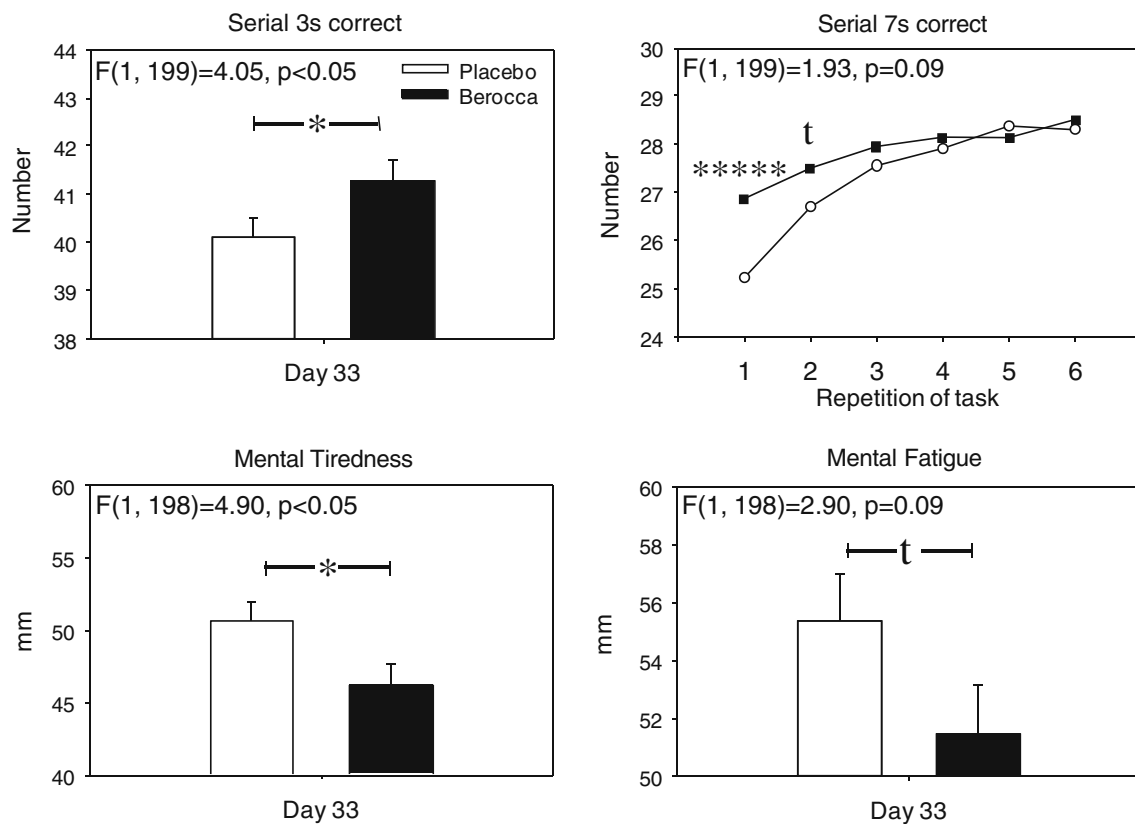Similarly the minerals zinc, magnesium and calcium have been highlighted as those most relevant to cognitive

Fig. 4 Significant effects of multi-vitamins/minerals on performance of the cognitive demand battery tasks, ratings of 'mental fatigue' during the battery and ratings of 'mental tiredness' measured before and after the battery. Data shown are adjusted means from two-way ANCOVAS (treatment × repetition) of day 33 data including averaged pre-treatment scores and fruit/vegetable consumption as covariates; *$p<0.05$, t $p<0.1$)

performance (Huskisson et al. 2007). Numerous nerve processes depend on calcium (Gareri et al. 1995) and it is a universal messenger of extracellular signals in a number of cells (Rasmussen 1986a, b). Magnesium plays an important role in a variety of metabolic reactions, particularly energy-requiring processes (Ryan 1991), and has been implicated in ensuring capacity for increased energy expenditure (Lukaski 2000). Zinc is essential to the structure and function of proteins and is highly concentrated in synaptic vesicles of a sub-set of glutamatergic neurons, which are particularly prevalent in the fore-brain (Frederickson et al. 2000). Neuropsychological impairment has also been highlighted as one of the major health consequences of zinc deficiency (Sandstead 2000).

The lack of data confirming pre-treatment dietary habits and circulating levels of the relevant vitamins/minerals is a limitation of the study and reduces the range of interpretations of the results. However, it is important to note that the two treatment groups did not differ in terms of cognitive task performance or their ratings on the various psychometric instruments employed in the study before treatment commenced, and given the randomised methodology and sample size, it seems reasonable to attribute the benefits

seen across a number of measures in the multi-vitamin/ mineral group to the treatment administered. Interestingly the groups did differ significantly on their self-reported consumption of fruit and vegetables (as per current definitions of 'portions'). However, this reflected greater consumption in the vitamins/minerals group. If this factor had any significant impact on the results, it might be conceived as reducing the liability of finding a positive result. The fact that including fruit/vegetable consumption as a second covariate marginally strengthens the results on two of the psychometric questionnaire measures, but does not modify the general tenor of the results, suggests that this factor may have a slight influence on the results seen here. However, it should be noted that there was no interpretable statistical relationship between this factor and scores on any measures pre-treatment across groups.

A further potential limitation of this study is that the consumption of vitamin B2 at the levels administered here can lead to discolouration of urine due to excretion of any excess. This could potentially have led to loss of blind in the current study. However, all participants (both those in the placebo group and the multi-vitamin/mineral group) were informed that ingestion of the investigational substance

could lead to a slight yellowish discolouration of urine and that this was harmless. No information was given that might indicate that this phenomena would be linked solely to the active treatment. Participants were also instructed to record and report any health observations/changes throughout the study period, and from a total of 210 participants, only 19 reported discolouration of urine, with four of these in the placebo group (Chi-square, non-significant). This is in keeping with previous findings (Carroll et al. 2000) and suggests that the participants remained blind to treatment condition throughout the trial.

In general, few studies have assessed the cognitive performance effects of vitamins/minerals in healthy, non-elderly cohorts. Whilst the merits of investigating focussed interventions in populations at risk of disease or age-related decrements in nutritional status and physiological parameters are obvious, a good case can also be made for conducting research with multiple micronutrients delivered in the combinations that healthy end consumers are likely to purchase in their everyday lives. Taken together with previous results showing beneficial effects of vitamin/mineral supplementation in healthy children (for reviews see Benton 2001; Eilander et al. 2010) and adults (Benton et al. 1995b; Carroll et al. 2000; Schlebusch et al. 2000), these findings further suggest that augmenting vitamin/mineral levels in healthy, normal populations may provide beneficial effects in terms of brain function. It is unclear whether these effects represent a an offset of impairment due to marginal deficiencies or an improvement due to sub-optimal levels that would not, under current guidelines, be classed as deficiency. However, given that a large section of the population are unable or unwilling to eat the adequately balanced diet that would satisfy their micronutrient requirements (Scientifc Advisory Committee on Nutrition 2008), it seems that supplementation with multi-vitamins/minerals may be a useful and possibly necessary option for this portion of the population.

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