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This is a copy of a paper originally published in the British Journal of Nutrition, 100 (5). pp. 1086-1096, November 2008.

British Journal of Nutrition (2008), **100**, 1086–1096
doi:10.1017/S0007114508959213

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(Received 29 May 2007 – Revised 13 February 2008 – Accepted 14 February 2008 – First published online 29 May 2008)

Adequate levels of vitamins and minerals are essential for optimal neural functioning. A high proportion of individuals, including children, suffer from deficiencies in one or more vitamins or minerals. This study investigated whether daily supplementation with vitamins/minerals could modulate cognitive performance and mood in healthy children. In this randomised, double-blind, placebo-controlled, parallel groups investigation, eighty-one healthy children aged from 8 to 14 years underwent laboratory assessments of their cognitive performance and mood pre-dose and at 1 and 3 h post-dose on the first and last days of 12 weeks' supplementation with a commercially available vitamins/mineral product (Pharmaton Kiddi™). Interim assessments were also completed at home after 4 and 8 weeks at 3 h post-dose. Each assessment comprised completion of a cognitive battery, delivered over the Internet, which included tasks assessing mood and the speed and accuracy of attention and aspects of memory (secondary, semantic and spatial working memory). The vitamin/mineral group performed more accurately on two attention tasks: 'Arrows' choice reaction time task at 4 and 8 weeks; 'Arrow Flankers' choice reaction time task at 4, 8 and 12 weeks. A single task outcome (Picture Recognition errors) evinced significant decrements at 12 weeks. Mood was not modulated in any interpretable manner. Whilst it is possible that the significant improvements following treatment were due to non-significant numerical differences in performance at baseline, these results would seem to suggest that vitamin/mineral supplementation has the potential to improve brain function in healthy children. This proposition requires further investigation.

Vitamins: Minerals: Cognitive performance: Attention: Children: Internet

Adequate levels of vitamins and minerals are essential for the optimal performance of a host of physiological processes that have both direct and indirect effects on brain function, including neurotransmitter synthesis, receptor binding, membrane ion pump function, energy metabolism and cerebral blood flow[1,2]. It is therefore unsurprising that a relationship has been shown to exist, in cross-sectional and prospective studies, between dietary consumption of vitamins and cognitive performance. Much of this research has focused on elderly populations, where positive relationships exist between cognitive performance and either dietary intake, or endogenous levels, of B vitamins[3–5] and vitamins C and E[6–8]. Previous intake of these vitamins has also been shown to be associated with a reduced risk of dementia[9].

The efficacy of direct supplementation with vitamins/minerals in terms of cognitive performance has received comparatively little attention. Evidence from the few studies in healthy adults that have included an assessment of elements of cognitive performance is somewhat equivocal[1] and this pattern is sustained in elderly cohorts[1,10–13]. However, it is notable that where cognitive measures have been included in adult and elderly studies they have tended to be secondary to other primary outcomes of the respective studies, rather than forming the focus of the investigation.

In the case of children, studies of supplementation with multi-vitamins/minerals have generally assessed measures of intelligence (or intelligence quotient) rather than cognitive performance *per se*. The balance of evidence here seems to suggest a propensity for improvement[1]. For instance, Benton's[14] review of the results of studies conducted in the previous decade notes that improved performance was seen in ten out of thirteen studies following supplementation, but with this restricted to non-verbal tests of intelligence. However, a recent article[15] from the Nutrition Enhancement for Mental Optimization study group concluded that fortification with multiple micronutrients can result in improvements in verbal learning and memory in well-nourished school-aged children.

Given that research suggests that a large proportion of the population are failing to consume adequate levels of specific vitamins in their normal diet[16–18] and that this situation is being exacerbated in many societies by a significant negative relationship between the consumption of 'fast food' and

Abbreviation: ANCOVA, analysis of covariance.

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vitamins[19], it seems timely to revisit the possibility that direct supplementation of children's diets with a multi-vitamin/mineral product will have a beneficial effect on cognitive performance and/or mood.

The current randomised, double-blind, placebo-controlled, parallel groups study therefore assessed the effects of a multivitamin/mineral supplement on cognitive performance and mood in a laboratory setting following 12 weeks' supplementation. In this case, the product under investigation was a widely available commercial supplement for children (Pharmaton Kiddi™), which has not previously been subjected to any similar investigation. The study utilised a purpose-programmed, Internet-delivered cognitive and mood assessment battery. The use of this novel assessment technique allowed further interim assessments to be undertaken in the participants' homes under parental supervision after 4 and 8 weeks of supplementation. There is also sparse but growing evidence that B, C and E vitamins are capable of positively influencing specific physiological processes which our group has previously argued may be relevant to cognition enhancement in the hours following single administration[20,21]. A secondary goal of the present study was therefore to determine whether micronutrient administration might have acute cognitive effects.

Methods and materials

Participants

A total of eighty-one male and female children aged 8 to 14 years took part in the study. They were recruited via posters and emails to university staff and students, from direct contact with schools in the surrounding area and further publicity via a newspaper article. All participants were reported to be healthy and free from any food allergy or the use of prescription, illicit, herbal or recreational drugs including alcohol and tobacco. Exclusion criteria also included the use of dietary supplements within the last 3 months and diagnosis with any significant medical condition. Exclusion/inclusion criteria were confirmed by questionnaire and interview. Prior to taking part in the study all participants and their parents provided written informed consent. The study was approved by the Northumbria University School of Psychology and Sport Science Ethics Committee and was carried out in accordance with the Declaration of Helsinki.

Following a blind review of the data, three participants were excluded on the basis that their data suggested that they had failed to perform the tasks correctly according to pre-determined criteria (i.e. performance across tasks that was at chance or consistent with use of a single button, plus large variations in reaction times suggesting inattention to the stimuli) on at least one occasion. Three sets of data from the day 1 post-dose assessments were also not captured due to a technical error. A further ten participants failed to complete one or both of the two home Internet assessments on the correct day/time. The blind data review also suggested that in a small number of instances individuals had mis-performed single tasks. Scores from these instances were excluded from the analysis.

Final datasets for analysis

In total, there were therefore seventy-five (placebo thirty-nine, verum thirty-six) complete datasets 'included' in the 'acute' analysis, sixty-eight datasets (placebo thirty-five, verum thirty-three) included in the 'interim' home Internet assessment and seventy-eight datasets (placebo forty, verum thirty-eight) included in the 'chronic' (full 12-week) analysis. Where the analysis for a single task represents data from less than these cohorts the number of participants contributing is shown in the respective table.

Demographic data from the seventy-eight children whose data was entered into the statistical analyses are presented in Table 1.

Treatments

Throughout the study period participants were asked to take two chewable tablets daily in the morning with breakfast. The treatments (placebo and vitamins/minerals) were provided by the manufacturer pre-coded according to a computer-generated randomisation list. All staff members at the investigational team remained unaware of the individuals' treatments until the completion of the blind data review.

During the laboratory visit days (days 1 and 85) the participants consumed their tablets at approximately 08.45 hours at the investigational site with their standard breakfast. On interim 'home' testing days (days 29 and 57), the participants were asked to take their study medication at their own home with their breakfast and to undertake the 'Internet testing battery' 3 h post-dose.

Participants were asked to take their day's tablets with their breakfast on every other day throughout the study period. The participant's parents were provided with a diary comprising a daily tick-box to initial as a means of confirming treatment consumption for the day.

Depending on which group the participant was randomly allocated to, the two chewable tablets comprised either Pharmaton Kiddi® multi-vitamins/minerals (see Table 2 for details of composition) or an inert placebo matching the verum tablets in all other respects.

Diary and Internet data suggested that all of the participants had 'good' (>80 %) compliance with regard to the consumption of daily treatments.

Cognitive and mood measures

Internet testing battery. The Internet battery comprised a selection of cognitive tasks programmed in Java language.

Table 1. Demographic data from the seventy-eight participants who contributed to the statistical analysis*

(Mean values and standard deviations)

	Vitamins/minerals		Placebo	
	Mean	SD	Mean	SD
Male (*n*)	19		16	
Female (*n*)	21		22	
Age (years)	10·84	2·57	11·26	2·02
Height (m)	1·46	0·13	1·50	0·13
Weight (kg)	41·07	12·54	43·83	12·42
BMI (kg/m^2)	18·93	4·57	19·28	3·61

There were no significant differences between treatment groups on these parameters.

* For details of subjects and procedures, see Methods and materials.

Table 2. Constituents of the multi-vitamin/mineral supplement (Pharmaton Kiddi®)*

Active ingredients	Dosage per tablet
L-Lysine monohydrochloride	50·00 mg
β-Carotene	0·514 mg
Vitamin A	Vitamin A: 715 IU
Thiamine nitrate	Vitamin B_1 nitrate: 0·500 mg
Riboflavin	Vitamin B_2: 0·550 mg
Pyridoxine hydrochloride	Vitamin B_6 hydrochloride: 0·550 mg
Cyanocobalamin	Vitamin B_{12}: 0·600 μg
Ascorbic acid	Vitamin C: 22·00 mg
Vitamin D_3	Vitamin D_3: 3·75 μg = 150 IU
Vitamin E acetate (d,L-α-tocopheryl acetate)	d,L-α-tocopheryl acetate: 5·215 mg = 5·22 IU d,L-α-tocopheryl acetate = 3·50 mg vitamin E
Folic acid	50 μg
Biotin	15·00 μg
Vitamin PP (nicotinamide)	6·00 mg
Copper(II)carbonate	Cu: 0·3 mg
Calcium phosphate (dibasic anhydrous)	Ca: 65·0 mg
Ferrous(II)fumarate	Fe: 2·50 mg
Zinc oxide	Zn: 2·50 mg
Magnesium oxide, heavy	Mg: 12·5 mg

* For details of subjects and procedures, see Methods and materials.

Timing of the test battery and reaction times were made independently of the computer's internal timing, guaranteeing consistent presentation of stimuli and accurate timing of responses. The presentation of parallel versions of stimuli for each individual task was counterbalanced across the assessments.

The battery included the following cognitive performance elements (cognitive domain in brackets where appropriate) and visual analogue mood items:

Word presentation: Fifteen words appropriate for the age range of the participants drawn from the Economic and Social Research Council's 'Children's Printed Word Database' and matched for familiarity, concreteness and frequency were presented at the commencement of the battery. Stimulus duration was 1 s, as was the inter-stimulus duration.

Picture presentation: Twelve age-appropriate line drawings of items (from Snodgrass & Vanderwart[22]) were presented at the commencement of the battery. Stimulus duration was 1 s, with a 2 s inter-stimulus duration.

Arrow reaction time test (choice reaction): An arrow appeared on the screen pointing to the left or right. Participants responded with a left or right arrow key press corresponding to the direction of the arrow. Each of the eighty stimuli remained on screen until the key press was registered. There was a randomly varying inter-stimulus interval of between 1 and 3 s. Outcomes were accuracy (i.e. % incorrect) and reaction time (ms).

Arrow Flankers test (choice reaction): Five symbols appeared on screen, with the centre symbol always being an arrow pointing to the left or right. The task was to press the right or left arrow key corresponding to the direction of the central arrow. The flanking pairs of symbols could be squares, crosses, congruent arrows (pointing in the same direction) or incongruent arrows (pointing in the opposite direction). Each of the eighty stimuli remained on screen until the key press was registered. There was a randomly varying inter-stimulus interval of between 1 and 3 s. Outcomes were accuracy (i.e. % incorrect) and reaction time (ms).

Paired associate learning (spatial working memory): Two shape symbols (e.g. square, circle, triangle, etc) were displayed on the screen side by side for 3 s. Each of the two symbols was then repeatedly presented alone in random order in the centre of the screen for a total of ten repetitions. The participant had to indicate if the symbol was originally seen on the left or right with a corresponding key press. A second pair of symbols was then presented and the four symbols that were contained in the two pairs were repeatedly presented (ten repetitions), with the participant once again indicating whether each symbol was originally presented on the left or right. This was repeated a further two times until responses were being made to one of eight symbols. Outcomes included the number of errors and reaction times (ms).

Sentence verification (semantic memory retrieval): Fifty short sentences appeared on screen that were either true (e.g. 'Bicycles have wheels') or false (e.g. 'Tomatoes have wings'). Participants responded 'true' or 'false' via a key press as quickly as possible. Outcomes included accuracy (% errors) and reaction times (ms).

Delayed word recognition (secondary memory): Word recognition was tested by the re-presentation of the fifteen words presented towards the beginning of the battery plus fifteen distractor words presented in random order. Participants responded either 'yes' or 'no' by key press to indicate whether the word had previously been presented. Outcomes included accuracy (% errors) and reaction times (ms).

Delayed picture recognition (secondary memory): Picture recognition was tested by the re-presentation of the twelve drawings presented at the commencement of the battery plus twelve distractor drawings presented in random order. Participants responded either 'yes' or 'no' by key press to indicate whether the picture had previously been presented. Outcomes included accuracy (% errors) and reaction times (ms).

Mood and fatigue visual analogue scales (mood): After completion of the cognitive tasks, participants were then asked to complete a computer-adapted version of the self-report visual analogue scales ('relaxed', 'alert', 'jittery', 'tired', 'tense', 'headache', 'overall mood') that have been used in previous research assessing dietary manipulations[23,24]. The scale was completed by using the computer mouse to place a cross on a line that represented a continuum between 'not at all' and 'extremely' for each of the seven mood items and a further three; 'mental fatigue' ('not at all' to 'extremely') and the bi-polar items 'Do you normally feel' 'happy/sad?' and 'stressed/calm?'.

Chalder fatigue scale. The Chalder Fatigue Scale[25] comprises eleven items, each of which can be scored out of four. These scores then aggregate into scores for physical and mental symptoms. In this instance, the scale was administered by the researcher in a verbal format, with the participant required to answer each of the eleven questions on a four-point scale ('better than usual'; 'no more than usual'; 'worse than usual'; 'much worse than usual').

Procedure

Participants attended the laboratory on three separate occasions at the weekend (practice day, day 1 and day 85). Testing took place in a suite of testing facilities with participants visually isolated from each other.

The practice day comprised of obtaining informed consent, training on the cognitive and mood measures and questionnaire measures, health screening, collection of demographic and nutritional data and random allocation to treatment. Participants also completed the Chalder Fatigue Scale at the end of the visit.

Following the practice day, participants attended the laboratory at 08.00 hours on the first (day 1) and last (day 85) days of the 12-week treatment regimen. On day 1, the participants received three bottles of chewable tablets. On arrival on day 1 and day 85, participants completed a baseline assessment of the Internet testing battery (see earlier). They then consumed one of a number of standard breakfasts that most closely corresponded to their habitually consumed breakfast, followed immediately by the day's treatment (08.45 hours). Given that the cognitive performance of children has previously been shown to deteriorate throughout the morning[26,27], and that this deterioration may serve to increase sensitivity to any intervention, two post-dose assessments were carried out using the Internet testing battery. These commenced at 1 h (09.45 hours) and 3 h (11.45 hours) post-treatment respectively. On day 85, the testing schedule was the same, with the exception that participants also completed the Chalder Fatigue Scale at the end of testing.

Interim assessments, comprising completion of the Internet testing battery, were also undertaken via the Internet in the participant's own home, under parental supervision, 3 h post-dose (participants were instructed to take their day's treatment at 08.45 hours with their breakfast) on days 29 and 57 via access to an individual web-address. Participants and their parents were e-mailed the day before Internet testing to prompt completion at the appropriate time.

The running order of the active testing sessions of the present study throughout the 85 d is shown in Fig. 1.

Following each of the laboratory assessments, participants also undertook a separate, secondary, methodological assessment comprising a number of measures designed for adults. These data are not reported for brevity. In all instances, the secondary data were always collected after the Internet battery.

Statistics

The data from the experiment were subjected to three separate analyses. The 'acute' analysis utilised the day 1 post-dose data collected in the laboratory. The 'interim' analysis utilised the day 29 and day 57 data collected over the Internet from the participants' own homes at 3 h post-dose. The 'chronic' analysis utilised the pre- and post-dose data collected on day 85 in the laboratory.

All of the separate analyses (with the exception of the Chalder Fatigue Scale data) were by repeated measures analysis of covariance (ANCOVA), with assessment as a repeated measures term, and condition as an independent measures term. To allow for any baseline differences that could impact upon results, baseline data collected pre-dose on day 1 were included as a covariate. Where significant condition × assessment interactions were found, these were explored with planned comparisons of data from the placebo and vitamin/mineral condition being carried out for each relevant assessment utilising *t* tests with the mean squares error from the ANCOVA as an error term and corrected means[28]. To ensure the overall protection level, all testing was two-tailed; comparisons were strictly planned prior to the study and only probabilities associated with these pre-planned comparisons were calculated.

In the case of the Chalder Fatigue Scale, a one-way independent measures ANCOVA of data from the laboratory visit on day 85 was carried out, utilising data from the practice day as a covariate.

Results

Acute (day 1) data analysis

There was a significant interaction between condition and assessment on Arrow Flankers reaction time ($F(1, 72) = 4{\cdot}15$, $P = 0{\cdot}045$) and accuracy ($F(1, 72) = 4{\cdot}10$, $P = 0{\cdot}047$). Planned comparisons at each time point revealed a significant decrease in speed of performing the Arrow Flankers task at 3 h ($t(72) = 2{\cdot}38$, $P = 0{\cdot}02$) following vitamins/minerals. At the same time point there was an improvement in accuracy (% errors) on this measure following the active treatment ($t(72) = 4{\cdot}04$, $P = 0{\cdot}0001$). There was also a significant condition × assessment interaction on accuracy of performing the Paired Associates task ($F(1, 71) = 5{\cdot}92$, $P = 0{\cdot}017$). Planned comparisons revealed that accuracy

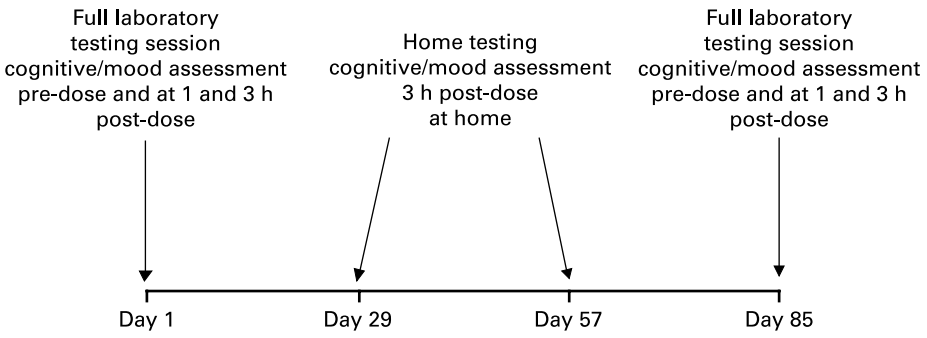

Fig. 1. The 12-week testing regimen showing laboratory and 'home' testing sessions.

on the Paired Associates task (number of errors) was significantly improved following vitamins/minerals at 3 h post-dose ($t(71) = 3{\cdot}43$, $P = 0{\cdot}001$). These results are represented in Fig. 2.

Interim (days 29, 57) data analysis

There was a main effect of treatment on the accuracy (% errors) of performing both the Arrows task ($F(1, 64) = 7{\cdot}22$, $P = 0{\cdot}009$) and the Arrow Flankers task ($F(1, 65) = 6{\cdot}30$, $P = 0{\cdot}015$), with improvements evident following vitamin/mineral administration.

There was a significant interaction between condition and assessment on 'jittery' ratings ($F(1, 65) = 5{\cdot}66$, $P = 0{\cdot}02$). Planned comparisons on each day revealed that these ratings were increased on day 29 ($t(65) = 3{\cdot}21$, $P < 0{\cdot}002$) following vitamins/minerals. These results are represented in Fig. 3.

Chronic (day 85) data analysis

Accuracy on the Arrow Flankers task was significantly improved following vitamins/minerals ($F(1, 75) = 4{\cdot}45$, $P = 0{\cdot}038$). Conversely, a significant decrement was seen following vitamins/minerals in terms of the accuracy of performing the Picture Recognition task ($F(1, 72) = 6{\cdot}79$, $P = 0{\cdot}011$).

There was a significant interaction between condition and assessment on 'jittery' ratings ($F(2, 150) = 4{\cdot}55$, $P = 0{\cdot}012$). Planned comparisons at each time point revealed that 'jittery' ratings for the vitamins/minerals group were reduced also pre-dose ($t(150) = 4{\cdot}52$, $P < 0{\cdot}001$). These results are represented in Fig. 4.

Unadjusted mean data for the acute, interim and chronic analyses of the cognitive and mood data are shown in Tables 3 and 4 respectively.

Chalder fatigue scale (day 85)

There were no significant differences on the Chalder Fatigue Scale.

Discussion

The most striking finding from the current study was a consistent pattern of enhancement across the attention task components of the battery, which was apparent throughout the assessments. The vitamin/minerals treatment improved accuracy of performance of the Arrows task during the day 29 and day 57 home assessments. A similar pattern was evinced in terms of accuracy on the Arrow Flankers task, although this effect was apparent 3 h after the first dose of vitamins/minerals (day 1) and was similarly sustained throughout both home testing sessions (days 29 and 57) and the day 85 laboratory assessment. It should, however, be noted that the effect on accuracy of the Arrow Flankers task on day 1 was coupled with slower reaction times on that task at the same time point (but not subsequently).

In contrast with these improvements in performance, micronutrient supplementation was associated with decrements in Picture Recognition (% errors) on day 85.

The two tasks that were consistently improved by supplementation rely, predominantly, on attentional resources. In this respect, the Arrows task is a straightforward 'choice reaction time' task assessing focused attention and the slightly more complex Arrow Flankers task has been described as assessing attention in the presence of distracting information. This would seem to argue for the administration of multivitamins/minerals having a specific beneficial impact on selective attention. Importantly, no task with a memory component was consistently enhanced by the treatment, again supporting a selective effect on attentional processing.

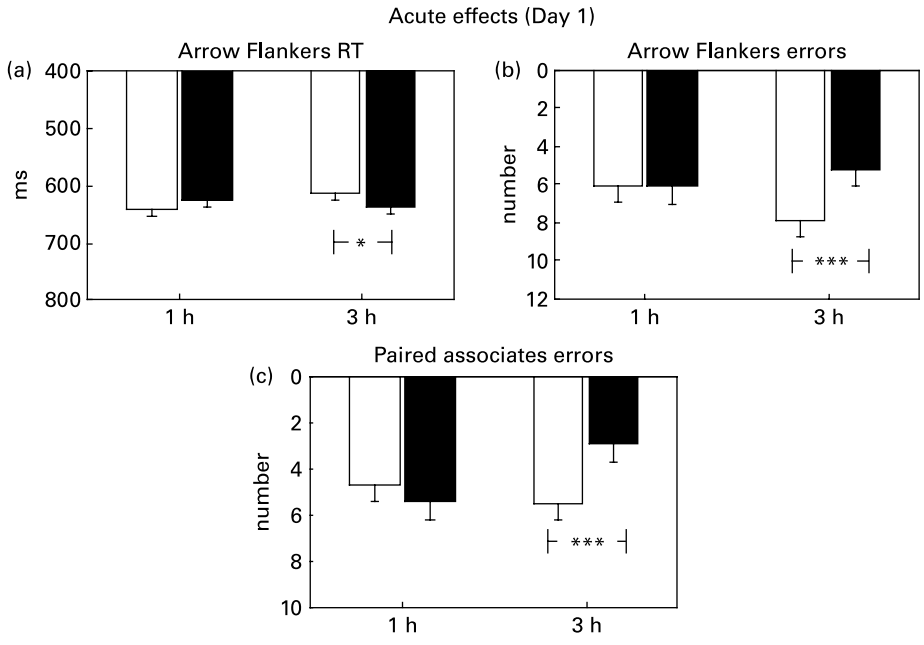

Fig. 2. Adjusted data for measures that generated a significant treatment × assessment interaction on the analysis of covariance performed on the acute data from 1 and 3 h post-dose on day 1 (*$P < 0{\cdot}05$; ***$P < 0{\cdot}001$; from planned comparisons between treatments at each time point). RT, Reaction time. □, placebo; ■, vitamins.

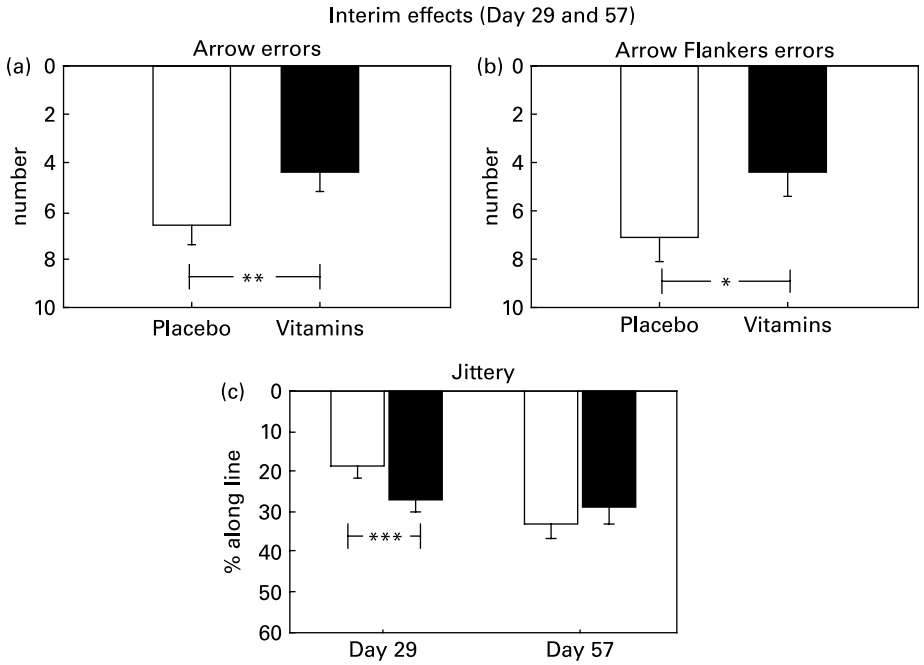

Fig. 3. Adjusted data for measures that generated a significant main effect (a,b) or treatment × assessment interaction effect (c) on the analysis of covariance (ANCOVA) performed on the home Internet data from 3 h post-dose on days 29 and 57 (*$P<0{\cdot}05$; **$P<0{\cdot}01$; ***$P<0{\cdot}005$ – (a,b) from the ANCOVA – (c) from the planned comparisons between treatments at each assessment). □, Placebo; ■, vitamins.

Interestingly, previous studies assessing the impact of vitamin/mineral supplementation in children have focused almost exclusively on the measurement of intelligence (intelligence quotient) rather than cognitive functioning *per se*. Whilst the findings from these studies are not entirely unequivocal (for reviews, see Haller[1] and Benton[14]), where significant differences have been found they have been positive and evinced on non-verbal, as opposed to verbal, aspects of intelligence. Benton[14] notes that verbal intelligence is a product of environment and experience and is a measure of specific

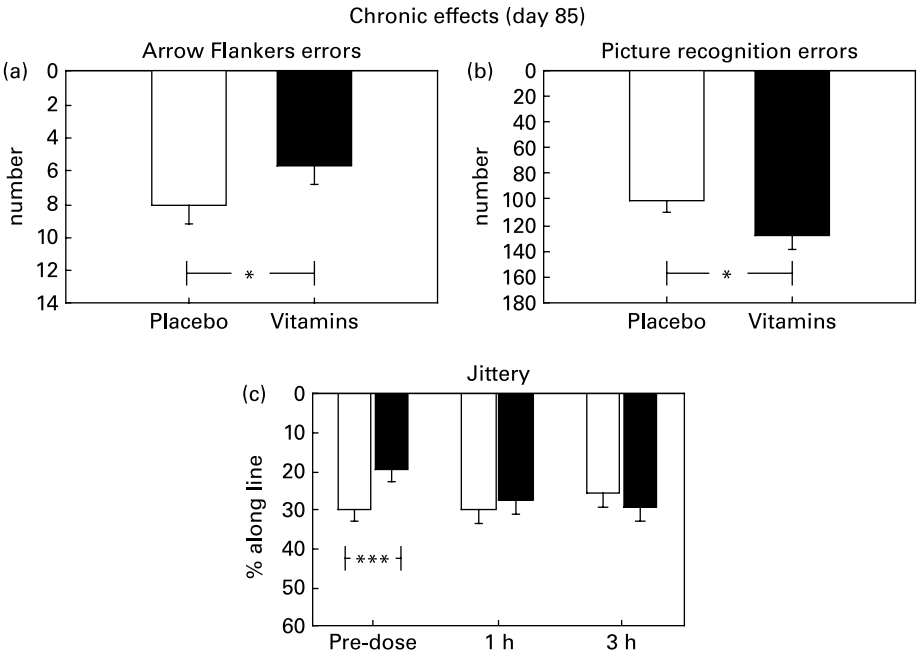

Fig. 4. Adjusted data for measures that generated a significant main effect (a,b) or treatment × assessment interaction effect (c) on the analysis of covariance (ANCOVA) performed on the chronic data from pre-dose, 1 h and 3 h on day 85 (*$P<0{\cdot}05$; ***$P<0{\cdot}005$ – (a,b) from the ANCOVA – (c) from the planned comparisons between treatments at each assessment). □, Placebo; ■, vitamins.

Table 3. Unadjusted cognitive task data from day 1 (acute analysis – *n* 75), days 29 and 57 (interim analysis – *n* 68) and day 85 (chronic analysis – *n* 78)‡
(Mean values and standard deviations)

Measure		Acute assessment (day 1)							Interim assessment (days 29 and 57)					Chronic assessment (day 85)						
			Baseline		1 h Post		3 h Post			Day 29		Day 57			Pre-dose		1 h Post		3 h Post	
		n 75	Mean	SD	Mean	SD	Mean	SD	*n* 68	Mean	SD	Mean	SD	*n* 78	Mean	SD	Mean	SD	Mean	SD
Arrows RT (ms)	Placebo	72	504	73	501	80	514	108	67	484	91	497	92	77	497	93	498	92	499	89
	Vitamins		504	64	516	118	522	114		506	92	505	81		501	76	505	84	520	101
Arrows (% error)	Placebo		4·3	3·9	8·0	6·0	8·6	6·1		4·9†	4·8	7·3†	6·7		4·9	5·2	10·9	9·5	11·7	10·7
	Vitamins		5·1	3·7	8·4	5·2	9·5	8·5		4·9†	3·7	4·7†	4·4		3·6	3·4	9·4	5·7	10·9	7·8
Arrow Flankers RT (ms)	Placebo		646	122	640	132	614	122		612	123	616	113		616	107	618	113	592	92
	Vitamins		644	85	623	88	635*	98		617	95	613	80		636	101	633	90	609	86
Arrow Flankers (% error)	Placebo		3·6	3·8	5·7	5·8	7·6	6·5		5·5†	4·4	7·8†	10·4		5·0†	5·4	8·4†	7·4	8·7†	9·3
	Vitamins		5·0	4·0	6·4	5·9	5·5*	4·4		5·2†	3·6	4·4†	3·5		5·7†	5·3	6·3†	6·6	7·5†	7·6
Paired Associates RT (ms)	Placebo	74	804	173	812	153	806	168	67	779	157	764	161		767	152	764	175	764	154
	Vitamins		782	141	768	123	779	163		751	162	772	120		784	140	802	173	794	176
Paired Associates (% error)	Placebo		2·8	3·2	4·8	4·8	5·6	5·8		2·7	3·1	2·3	2·1		2·6	3·0	4·3	4·0	3·9	3·4
	Vitamins		2·3	3·3	5·3	4·3	2·8*	2·1		2·9	3·9	3·1	2·6		2·4	2·3	3·9	2·8	4·3	4·9
Sentence Verification RT (ms)	Placebo	72	2079	699	2007	732	1903	723	67	2063	1190	1986	1057	77	2022	1012	1959	1191	1760	769
	Vitamins		2375	887	2227	898	2129	786		2150	801	2197	993		2183	903	2031	886	2077	1050
Sentence Verification (% error)	Placebo		9·0	6·9	9·4	6·4	11·9	7·9		9·6	7·1	9·0	7·8		9·3	6·7	10·6	9·9	12·9	12·4
	Vitamins		12·2	10·5	13·2	10·2	13·7	9·9		10·2	8·6	11·3	9·1		11·3	8·5	13·6	9·8	13·3	11·0
Picture Recognition RT (ms)	Placebo		988	220	929	205	970	422	59	919	510	801	212	75	871	188	852	151	856	189
	Vitamins		960	281	904	204	953	287		864	328	962	365		1003	323	867	242	885	370
Picture Recognition (% error)	Placebo		7·1	0·9	12·4	1·4	14·7	1·3		11·8	2·1	8·8	1·4		4·5†	0·6	13·4†	1·2	14·2†	1·1
	Vitamins		5·4	1·0	13·5	1·6	15·9	1·6		14·6	2·2	9·7	1·7		6·1†	1·0	14·3†	1·4	16·5†	1·5
Word Recognition RT (ms)	Placebo	74	882	181	794	199	787	233		814	258	775	195	77	835	205	786	241	767	242
	Vitamins		887	178	864	220	836	207		845	212	864	269		860	194	814	212	812	301
Word Recognition (% error)	Placebo		16·4	1·4	21·6	1·6	20·3	1·4		17·2	1·3	17·1	1·5		16·2	1·1	20·0	1·1	22·0	1·3
	Vitamins		16·4	1·3	19·8	1·3	21·1	1·5		15·6	1·5	14·3	1·7		15·6	1·4	20·7	1·3	22·3	1·2

RT, reaction time.
* Significant planned comparison ($P < 0·05$) between treatments on measure that generated a treatment × assessment interaction.
† Significant main effect of treatment across assessments ($P < 0·05$; analysis of covariance).
‡ Where a specific task resulted in a reduced dataset the number of participants contributing data to the specific analysis is shown within the Table. For details of subjects and procedures, see Materials and methods.

Table 4. Unadjusted mood visual analogue scale data from day 1 (acute analysis – *n* 75), days 29 and 57 (interim analysis – *n* 68) and day 85 (chronic analysis – *n* 78)†
(Mean values and standard deviations)

		Acute assessment (day 1) (*n* 75)						Interim assessment (day 29 and 57) (*n* 68)				Chronic assessment (day 85) (*n* 78)					
		Baseline		1 h Post		3 h Post		Day 29		Day 57		Pre-dose		1 h Post		3 h Post	
Measure		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Relaxed	Placebo	54·23	21·00	63·1	17·4	62·0	23·5	62·1	21·0	60·2	18·3	56·2	19·3	60·8	21·0	67·2	21·3
	Vitamins	61·64	19·52	63·5	23·6	64·1	20·6	64·0	22·3	61·9	26·6	59·9	23·5	62·0	25·9	60·1	27·9
Alert	Placebo	40·38	22·03	54·6	23·0	57·0	22·6	54·2	25·1	61·7	19·7	52·7	20·6	55·0	23·2	57·5	22·8
	Vitamins	50·53	20·85	56·8	25·3	60·1	21·0	64·7	20·8	65·2	24·9	50·6	27·3	59·7	25·7	58·6	24·3
Jittery	Placebo	27·82	20·18	27·0	20·7	29·9	20·4	19·0	18·1	33·3	22·7	30·4	23·9	30·7	25·6	26·3	21·6
	Vitamins	26·11	19·27	32·0	25·4	28·9	22·3	26·6*	22·1	28·5	26·4	18·9*	17·9	26·5	21·9	28·3	24·8
Tired	Placebo	63·03	24·79	44·3	27·1	42·2	27·0	45·9	25·7	38·9	20·6	60·3	22·6	48·5	23·0	44·1	24·9
	Vitamins	52·56	26·45	33·0	26·0	34·5	24·1	34·0	26·6	32·3	24·3	50·1	30·5	36·0	26·9	36·0	27·8
Tense	Placebo	30·31	16·98	30·9	22·3	27·5	21·7	26·3	21·4	29·1	21·9	35·3	26·0	30·3	24·2	28·4	23·1
	Vitamins	31·31	22·57	28·5	17·7	28·3	18·1	22·3	19·2	25·7	24·3	24·7	22·5	26·4	21·8	30·3	23·1
Headache	Placebo	17·44	24·08	16·4	21·8	17·1	24·0	18·0	26·6	16·3	24·3	20·6	28·0	18·1	21·9	23·4	25·7
	Vitamins	16·11	20·79	12·3	16·2	15·0	20·0	18·5	23·6	19·1	25·7	19·9	25·6	17·6	21·8	20·9	23·5
Overall mood	Placebo	68·33	15·97	77·1	14·9	71·8	21·9	76·1	18·7	75·5	18·3	72·6	17·6	74·3	15·2	76·5	19·9
	Vitamins	69·47	19·06	77·8	17·5	74·8	18·8	70·8	25·4	73·7	18·4	69·4	22·9	71·8	21·4	73·9	20·1
Mentally fatigued	Placebo	40·31	20·12	40·4	21·0	41·5	26·6	33·6	24·3	31·2	22·1	37·8	24·2	30·7	18·7	34·3	23·4
	Vitamins	37·25	19·73	39·9	24·0	36·1	26·0	30·3	23·0	32·7	28·9	32·4	24·3	31·9	28·0	31·8	25·2
Happy/sad	Placebo	19·92	15·21	19·0	18·2	18·2	18·9	17·3	16·2	19·9	17·1	19·0	17·4	17·4	18·7	17·7	17·4
	Vitamins	20·58	17·03	20·0	16·7	19·7	16·6	25·9	21·3	22·2	21·0	18·0	16·5	15·1	16·4	17·7	18·4
Stressed/calm	Placebo	73·33	18·70	71·0	21·9	73·1	24·7	71·1	21·3	70·5	20·6	72·2	22·0	75·0	17·5	74·1	19·1
	Vitamins	64·36	23·29	65·2	25·6	64·2	23·7	66·8	22·5	63·9	25·7	68·5	25·6	72·6	26·4	72·9	27·2

* Significant planned comparison ($P < 0·05$) between treatments on measure that generated a treatment × assessment interaction.
† For details of subjects and procedures, see Methods and materials.

information and vocabulary, whereas non-verbal intelligence reflects basic problem solving and reasoning power. Any supplementation that had an effect on brain biochemistry would therefore be expected to impact non-verbal intelligence as this domain reflects simple 'biological potential'[14]. Whilst the results here are broadly in line with this differentiation, it is also notable that any improvement in measures of 'non-verbal intelligence' previously seen following vitamins/minerals could also be more parsimoniously explained by a simple improvement in general attention, as possibly indicated by the present results.

The most surprising facet of the improvement in attention task performance seen here is that it became evident by 3 h post-dose on the first day of treatment. Although it is possible that this effect merely reflects a speed–accuracy trade-off, given that reaction time was significantly slower at the same time point, this effect was also accompanied by a single significant improvement in the accuracy of the Paired Associate task at the same time point. To the best of our knowledge, the possibility that vitamins or minerals could exert behavioural effects after a single dose has not been explored. Nonetheless, there is emerging evidence that micronutrient supplementation may have acute effects upon cognition-relevant physiological processes within the same time-frame as the effects observed here. For example, various chronic and acute disruptions to endothelial function can be reversed by acute administration of micronutrients. Thus, 5 mg folic acid improved flow-mediated dilatation at 2 and 4 h post-administration in coronary artery disease patients – an effect that was maintained following 6 weeks supplementation[29]. The same parameter is reduced during and in the hours and days following underwater diving. This measure was selectively improved after diving in healthy professional divers who had been administered 2 g vitamin C with 400 IU vitamin E 2 h prior to the dive (of unspecified duration)[30]. Nutritional loads can also negatively impact upon endothelial function. Co-administration of 800 IU vitamin E prevented the disruption to endothelial function (here assessed by brachial artery post-occlusion peak flow) associated with fat ingestion measured 3 h post-administration[31]. Similarly 2 and 3 h preloading with a combination of 2 g vitamin C and 800 IU vitamin E attenuated the reduction in endothelial function associated with a 75 g glucose load[32]. We have previously argued that interventions that improve the central delivery of metabolic substrates are likely to act as cognition enhancers[20,21]. However, these studies used doses approximately two orders of magnitude higher than those in the current trial and had very different (non-cognitive) outcomes in different populations. Given the paucity of data regarding acute effects of micronutrient administration, it would be unwise to attribute any of the effects observed here to endothelial or other effects. Nevertheless, such findings argue strongly for further studies into acute effects of micronutrient supplementation.

Although the mechanisms underlying these putative effects are unknown and a detailed discussion of potential processes involved is beyond the scope of this empirical paper, the reader is directed to a recent review[2] for a detailed consideration of mechanisms relevant to brain health and cognitive function. In this review, the authors describe direct and indirect modulation by micronutrient supplementation of major neurotransmitter and neuromodulator systems. These include (but are not restricted to) influences via amino acid modulation on GABAergic, serotonergic, dopaminergic, adrenergic and histaminergic pathways.

Whilst the pattern of results across the attention tasks is remarkably consistent, with effects evinced across the acute, interim 'at home' and chronic assessments, the decrements seen on the Picture Recognition task (% errors) were evinced solely during the chronic (day 85) laboratory session. The factors underlying this decline in performance are difficult to delineate. In general, this and the Word Recognition task were poorly performed, with many participants barely exceeding chance performance on the later assessments on day 85. The task was also positioned late in the battery. The significant difference may therefore represent an interaction between the treatment and task demands and/or waning attention. This interpretation would also raise the question as to whether the attention task improvements were domain specific, or simply the result of these tasks being first in the battery. Alternatively, in the absence of any other significant decrements during either the acute or interim assessments on the Picture Recognition task, or indeed the other memory tasks within the battery, it is possible that this finding simply represents a chance fluctuation in performance (type I error).

Similarly, the interactions on the 'jittery' mood scale (with an increase on day 29, but a decrease pre-dose on day 85) are also difficult to interpret. However, it may be relevant that this scale was included simply on the basis that it was contained within the set of visual analogue scales utilised here[24,25] and was generally not well understood by the children. Indeed 'jittery' had to be explained to the vast majority of the cohort by the researchers. This suggests that, again, these interactions may simply represent type I errors as a consequence of fairly random variations due to a lack of understanding of the question.

Similarly, it should be noted that any statistical technique that uses a measure of pre-treatment baseline performance as a yardstick against which to measure any subsequent treatment-related effect (as the ANCOVA did in this case) runs the risk of a between groups difference in baseline, covariate performance contributing to, or negating, the effects of the treatment by a subsequent regression to the mean. In this case, there were numerical, but non-significant, differences in the scores for placebo on the measures of attention that subsequently evinced the pattern of significant enhancement post-dose. These pre-dose differences could suggest two further interpretations rather than the straightforward 'net benefits' proposal made earlier. The first is that the numerical, but non-significant, pre-dose differences represent a chance fluctuation in performance that led to, or contributed to, the significant post-dose differences in scores. However, this proposition would suggest that all of the post-dose scores, including both post-dose assessments on day 1, should be similarly and equally modulated (as they all have the same pre-dose covariate score). This was patently not the case, with the Arrows Flankers task evincing a treatment × assessment interaction (i.e. no difference at 1 h post-dose, but an improvement at 3 h) on day 1, and the somewhat less demanding Arrows task only showing improvements that became apparent after 29 d. The second alternative interpretation would be that the treatment had attenuated a genuine decrement in performance

in the 'vitamin group'. However, it should be stressed that there was no evidence of a significant pre-dose difference in the groups. The multiple potential interpretations of the results seen here do, however, support the assertion that the effects of supplementation of this nature require further investigation.

The use of a cohort of healthy children, who were not selected on the basis of an examination of their dietary habits, was either a strength, or alternatively weakness of the study, depending on one's view point. Whilst the lack of fine-grained dietary information precludes an assessment of the comparative effects of vitamins/minerals in children with different nutritional backgrounds, the use of a simple cross-section does seem to suggest that the results can be generalised to relatively normal populations. That having been said, there is also nothing to suggest that the children under investigation did not include the high proportions reported to be deficient in one or more micronutrients[16–19]. It should be noted that an analysis of BMI for the participants in the current study revealed that 26 % were overweight (as classified in Cole *et al.*[33]). However, this level is in line with figures reported by the International Obesity Task Force in 2004 for UK children.

The combination of vitamins, minerals and amino acids present in the active treatment in the present study does not allow the results presented to be attributed to any one component. However, the product tested is available internationally and is aimed at improving physical development and neural performance. This claim clearly requires validation and the primary aim of the current study was to ascertain whether claims made by the manufacturers with regard to potentially improved mental performance following administration have any basis in fact. Further work in this area could examine the constituent parts of this treatment in more detail, perhaps focusing on attentional measures and including acute, as well as chronic, assessment.

In conclusion, the results of the current study demonstrated a consistent improvement in the accuracy of attention task performance, in comparison with placebo, during 12 weeks' supplementation with vitamins/minerals in healthy 8- to 14-year-old children. Furthermore, the first signs of any effect were detected 3 h following the first dose of vitamins on day 1 of the study. This represents the first observation of acute behavioural effects of vitamins/minerals in human subjects. Naturally, these observations require replication in larger cohorts, but they do suggest that this matter should be given some priority.

Acknowledgements

The research project described herein was made possible by funding and materials provided by Pharmaton SA, Lugano, Switzerland. However, none of the authors has any financial interest, either directly or otherwise, in Pharmaton SA or any of their products and therefore feel there is no conflict of interests.

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