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Improved cognitive performance following supplementation with a mixed-grain diet in high school students: A randomized controlled trial

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ABSTRACT

Objective: Adolescence is a stage of rapid growth, when rich nutritional supplementation is important. Maintaining optimal cognitive functioning is critical in high school students, who are under considerable academic pressure. The objectives of this study were to identify the effects of a 9-wk randomly assigned diet of mixed grains versus a regular diet on cognitive performance and on levels of plasma brain-derived neurotrophic factor (BDNF) and S100B, a calcium-binding protein produced by astroglial cells, in healthy high school students (grades 10 and 11).
Methods: In this 9-wk, single-blind, controlled study, subjects were randomly allocated to either a mixed-grain or a regular diet. Cognitive assessments and measurements of plasma BDNF and S100B levels were performed at baseline and after the 9-wk intake of a mixed-grain or regular diet. Computerized neuropsychological tests and self-rating scales were used for the cognitive assessments.
Results: Significant improvements in some neuropsychological tests were found after 9 wk in both the mixed-grain and the regular-diet groups, but the changes from baseline between the two groups were not significantly different. Significant impairments on the AX-continuous performance test were observed at the endpoint in the regular-diet group, and the changes from baseline between the two groups were also significantly different for this test. A significant difference in changes in BDNF levels was observed between the two groups.
Conclusions: These results suggest that intake of mixed grains for 9 wk is beneficial for cognitive performance and plasma BDNF levels in high school students. These beneficial effects seem to be related to the prevention of cognitive deterioration in a mental-fatigue test with the mixed-grain diet, rather than cognitive enhancement per se.

Introduction

The effects exerted by food on cognition and behavior are increasingly being recognized.

A good deal of evidence suggests that intelligence scores can be improved by micronutrient supplementation in children and adolescents with very poor dietary status, and missing breakfast may have negative consequences late in the morning [1–3]. The effects of food or nutrients on cognitive function have been mainly investigated in infants or children, when rapid brain development occurs. However, evidence suggests that brain maturation continues throughout adulthood. Specifically, several significant changes occur during adolescence in the prefrontal cortex, including loss of gray matter density [4], increases in white matter volume [5] and cerebral blood flow [6], and synaptic pruning [7,8]. These changes parallel the development of higher order cognitive functioning during adolescence, such as deductive

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reasoning, planning, executive functioning, and metacognition. Adolescence is a period of rapid growth, development, and hormonal change, accompanied by increased academic and peer pressure. In Korea, high school students are under a great deal of academic stress because of fierce competition for college places. The combination of brain maturation in the prefrontal cortex and significant academic pressure makes great demands on adolescents, so it could be beneficial to provide a diet that helps them to optimize their cognitive functioning. There is, however, a paucity of research on the effect of nutrition on cognitive functioning in adolescents. Several studies have reported positive associations between food insufficiency and academic achievement or cognitive performance: academic achievement has been positively correlated with frequency of consumption of dairy, meats, and eggs [9]; food insufficiency has been significantly associated with arithmetic scores [10]; and a significant association has been found between skipping breakfast and poor school performance [11].

Compared with polished rice, brown rice has many additional nutrients such as vitamins, minerals, and fiber, making it attractive as a healthy functional food. Malting cereal seeds has long been used to improve the bioavailability of nutrients. The malting process, including steeping and germination, is reported to be effective for stimulating the production of γ-aminobutyric acid (GABA), amino acids, and monosaccharides in brown rice [12]. Among the produced metabolites, GABA is of particular interest because of its health-promoting impacts, such as decreasing blood pressure [13], preventing alcohol-related disease [14], and inhibiting cancer-cell proliferation [15]. Additionally, it has been reported recently that a partial inverse agonist acting at α_5-$GABA_A$ receptors enhanced the performance of wild-type rats in a water maze test [16] and reduced ethanol-induced memory deficit in healthy volunteers [17], supporting the important role of GABA in learning and memory. However, no study has been undertaken about the cognition-enhancing effects of germinated brown rice or GABAergic drugs in healthy volunteers. DeungRyong Rice Processing Complex (Buan, Korea) has developed a mixed-grain product composed of germinated brown non-glutinous rice with giant germ, germinated brown glutinous rice, non-glutinous polished rice with giant germ, non-glutinous black rice, kidney beans (puffed grit), and walnuts. The present study investigated the effects of a mixed-grain product on the cognitive functioning of high school students in Korea. Our hypothesis was that long-term intake of this mixed-grain product would either enhance cognitive functioning or prevent cognitive deterioration during cognitively demanding tasks, i.e., mental fatigue tests. We also measured plasma brain-derived neurotrophic factor (BDNF) and S100B (a calcium-binding protein produced by astroglial cells) levels, biological markers implicated in synaptic plasticity and memory processes, to see if their levels were affected by intake of the mixed-grain product.

Materials and methods

Subjects

Participants were aged 15-17 y, were all in grades 10 and 11 of the same high school, and gave informed consent to take part in the present study. We restricted our sample to males living in a dormitory to control for endocrinologic influences on cognitive tests and for easy diet control, respectively. Entry criteria were having no significant psychiatric, neurological, or medical illnesses and no significant laboratory findings; being within ±30% of ideal body weight; not consuming any micronutrient supplements or tonifying herbal medicines during the 2 wk preceding the start of the study; and not intending to use these supplements or herbal medicine during the course of the study. To screen for significant psychiatric problems, psychiatric interviews were performed by a psychiatric trainee (CH) using the screening module of the Structured Clinical Interview for DSM-IV-NP [18,19]. Written informed consent was obtained from the parents of the participants, and oral assent was obtained from the participants. The Institutional Review Board of the Clinical Trial Center for Functional Foods and Chonbuk National University Hospital approved the study protocol; the approval number was 2009-02-004.

Study design

This was a 9-wk, single-blind, randomized, controlled, parallel experiment. During the screening period, a physical examination, medical history, basic laboratory tests, and psychiatric interview were performed for each participant. For eligible individuals, data for cognitive tests and self-rating scales and blood sampling for BDNF and S100B were obtained at baseline and after the 9-wk treatment period. Participants were then randomly allocated to either a mixed-grain-product diet or a regular diet. The random assignment was carried out using a Latin square design. The dietary interventions began immediately after baseline data collection. Participants who successfully conformed to the study regulations were rewarded with an MP3 player near the end of the study.

Interventions

The compositions and nutrients of the mixed-grain and regular diets are provided in Tables 1 and 2. The mixed-grain product was composed of germinated brown non-glutinous rice with giant germ, germinated brown glutinous rice, non-glutinous polished rice with giant germ, glutinous black rice, kidney beans, and walnuts. The regular diet was non-glutinous polished rice. The calories for the mixed-grain and regular diets were 411.52 and 417.60 kcal, respectively. Proximate composition and dietary fiber content were determined using the Association of Official Analytical Chemists official methods [20]. GABA content was analyzed by an automated amino acid analyzer (S4300; Sykam Co., Eresing, Germany) using the ninhydrin method [21]. Vitamin B complex content was analyzed by high-performance liquid chromatography systems (Shiseido Nanospace S12; Shiseido Co., Tokyo, Japan) using the Korea Food and Drug Administration method [22] and Marszall et al.'s method [23]. Mineral content was analyzed by the wet-ash method [24] using an atomic absorption spectrometer (Solaar M5; Thermo Elemental Co., Cambridge, UK) and spectrophotometer (UV-1601; Shimadzu Co., Kyoto, Japan).

Participants received 120 g of mixed-grain product or regular diet at each meal three times per d for 9 wk, and they were asked not to leave leftovers or eat snacks between meals. When they stayed at home (usually once a month), wrapped meals were provided for them. They were asked to record their food intake and to maintain their usual physical activity and lifestyle during the study. Taking micronutrient supplements or herbal medicines was strictly forbidden. Compliance, body weight, and waist circumference were checked weekly by trained research assistants.

Measures

Cognitive tests

The Computerized Neuropsychological Test (CNT) 40 (Maxmedica, Inc., Seoul, Korea) was used to assess cognitive functioning. This test battery included the auditory continuous-performance test (A-CPT), digit-span test (DST), Stroop test, trail-making tests A and B (TMT A and B), verbal-learning test (VLT), Wisconsin Card Sorting Test (WCST), and the Thurstone Word-Fluency Test (TWFT). The A-CPT, DST, and Stroop test are attention tests; TMT B and WCST assess executive functioning; VLT tests memory; the word fluency test measures language; and TMT A and B evaluate visual–motor speed. To induce mental fatigue, two repetitions of a rapid visual information-processing task (RVIP) (first and last, each 5 min) and one repetition of AX-CPT (middle, 60 min) were

Table 1
Compositions of the mixed-grain and regular diet

Compositions	Mixed grain (%)	Regular diet (%)
Giant germ rice	33.3	
Germinated giant germ brown rice	28.3	
Germinated glutinous brown rice	18.3	
Non-glutinous black rice	3.3	
Kidney beans (puffed, grits)	12.5	
Walnut	4.2	
Polished rice		87.5
Glutinous rice		7.8
Barley		1.2
Glutinous brown rice		1.2
Glutinous sorghum		1.2
Glutinous black rice		1.2
Total	100.0	100.0

Table 2
Nutrients (mg or g/100 g) of the mixed-grain and regular diet

Nutrients	Mixed grain	Regular diet	% (mixed–grain/ regular diet)
Protein (g)	10.6	6.8	155.1
Fat (g)	3.6	1.9	192.0
Carbohydrate (g)	74.1	78.7	94.3
Total dietary fiber (g)	13.4	6.5	207.9
Soluble dietary fiber (g)	1.5	1.1	141.0
Insoluble dietary fiber (g)	11.9	5.4	220.9
γ-Aminobutyric acid (g)	5.3	0.8	627.4
P (mg)	293.8	88.7	331.4
Na (mg)	5.0	6.9	72.2
K (mg)	210.2	46.6	451.1
Cu (mg)	0.4	0.2	182.4
Zn (mg)	2.1	1.3	161.5
Fe (mg)	3.0	2.2	139.4
Ca (mg)	29.7	6.6	450.5
Mg (mg)	86.4	13.7	630.7
Vit B1 (mg)	0.2	0.1	200.0
Vit B2 (mg)	0.2	0.2	100.0
Vit B3 (mg)	2.8	0.3	951.7
Vit B6 (mg)	0.2	0.0	300.0

consecutively administered. In the AX-CPT, sequences of letters were visually presented one at a time in a continuous fashion on a computer screen. Participants sat in front of a response box and were instructed to press the right button on target trials and the left button otherwise. Target trials were defined as a cue-probe sequence in which the letter A appeared as the cue and the letter X appeared as the probe. The remaining letters of the alphabet served as invalid cues and non-target probes, with the exception of the letters K and Y, which were excluded because of their similarity in appearance to the letter X. Letter sequences were presented in pseudorandom order, such that target (AX) trials occurred with 70% frequency, and non-target trials occurred with 30% frequency. Non-targets were divided evenly (10% each) among the following trial types: BX trials, in which an invalid cue (i.e., non-A) preceded the target; AY trials, in which a valid cue was followed by a non-target probe (i.e., non-X); and BY trials, in which an invalid cue was followed by a non-target probe. To increase task difficulty, two white distracter letters (which could be any letter except A, K, X, or Y) were presented between the cue and probe, which were both red. All letters were presented centrally on a black background for 300 ms. Each letter was followed by a 1200-ms interval, which gave a 4500-ms delay between the presentation of cue and probe stimuli. The dependent variables were numbers of correct and incorrect responses and omission errors in target and non-target trials, and a sensitivity index. In the RVIP task, participants monitored a continuous series of digits for targets of three consecutive odd or three consecutive even digits. The digits were presented on the computer screen at a rate of 100 per min in pseudorandom order, and the participant responded to the detection of a target string by pressing the space bar as quickly as possible. The task was continuous and lasted for 5 min, with eight correct target strings being presented during each minute. The task was scored for number of target strings correctly detected, average reaction time for correct detections, and number of incorrect responses. CNT 40 and mental-fatigue tests were conducted at both baseline and endpoint, but on different days to prevent mental exhaustion from extended testing. The cognitive tests were conducted in the evenings between 8 pm and 10 pm, about 2 h after dinner. It took about 40 min and 1.5 h to complete the CNT and mental-fatigue tests, respectively. The cognitive assessments were conducted by experienced research nurses who were blind to the objectives of the study.

Self-rating scales

To assess subjective changes in cognitive functioning, seven-point (1-7) Likert scales for concentration, comprehension, executive function, memory, persistence, task-processing speed, and verbal expression were used: 1-3 indicated below usual status; 4 was usual state; and 5-7 indicated above usual status. The Stress–Arousal Checklist (SACL) [25] was administered to measure current perceived stress and arousal. The SACL has 30 items (four-point Likert scale) consisting of two subscales, one to determine self-reported stress (16 items) and one to determine self-reported arousal (14 items), with a higher score in each domain meaning a higher level of stress or arousal, respectively. These two self-rating scales were also administered at baseline and endpoint.

Laboratory tests and BDNF and S100B assay

During the screening period and after the 9-wk treatment, basic laboratory tests, including blood cell count, electrolytes, liver function test, urinalysis, chest X-ray, and electrocardiogram, were performed. For BDNF and S100B assays, a blood sample (16 mL) was drawn from the cubital vein in EDTA-coated tubes between 7 am and 9 am after overnight fasting to minimize the effects of possible circadian variations at baseline and endpoint. The tubes were kept on ice after collection, and blood samples were immediately centrifuged at 4°C (3000 rpm for 10 min). Plasma was aliquoted and stored at −80°C. Plasma BDNF was determined using an enzyme-linked immunosorbent assay (ELISA) (Quantikine Human BDNF immunoassay kit; R&D Systems, Minneapolis, MN, USA). S100B was also determined by ELISA (Human S100B ELISA; BioVendor Laboratory Medicíne, Inc., Candler, NC, USA). The detection range of the assay for BDNF was from 20 to 4000 pg/mL. The assay's lower detection limit for S100B was 15 ng/L. All samples were analyzed in triplicate in one session by an investigator blind to the experimental setup.

Statistical analysis

For demographic variables, descriptive statistics were calculated. Data were analyzed using a two-way (treatment × time) repeated-measures analysis of variance (ANOVA). For all other variables, within-group analyses were performed using paired *t*-tests. Changes in measurements over the 9-wk study period were obtained by calculating the difference between the pre- and postintervention measurements in each group. Significance was assessed by comparing changes over the 9-wk study period in the mixed-grain and control groups using each participant's *t*-tests. All data are presented as means with standard deviations. A criterion of $P < 0.05$ was set for statistical significance.

Results

The 28 enrolled subjects were randomized equally to mixed-grain or regular-diet groups. Two subjects in the regular-diet group were excluded because of possible influenza, a viral (H1N1) infection, commonly called swine flu. Baseline characteristics, including age and body weight, did not differ between the groups (Table 3). Vital signs and laboratory tests at baseline revealed no abnormal findings in any participants, and no significant differences were observed between the changes in two groups after the treatment except in red blood cell count and in aspartate aminotransferase (AST) and alanine transaminase (ALT) levels (Table 4). These significant changes were clinically non-significant. The compliance was 97% and 95% for mixed-grain and regular diet groups, respectively.

No significant treatment × time interactions were found in any of the variables. The CNT 40 (Table 5) revealed significant improvements in the VLT and TMT B in both the mixed-grain and the regular-diet groups after 9 wk. However, no significant differences were observed between the change scores of the two groups except in the change scores for delayed recall at 20 min, which was higher in the regular-diet group compared with the mixed-grain group. In the AX-CPT mental-fatigue tests (Table 6), the regular-diet group showed a significant decrease in correct responses and an increase in omission errors at the endpoint compared with baseline. Furthermore, significant differences between the two groups were observed in the changes in correct responses and omission errors in the AX-CPT. The self-rating scale for cognitive functioning (Table 7) revealed significant improvements at the endpoint compared with baseline in both groups, including improvements in executive function, task processing speed, and total score in the mixed-grains group, and

Table 3
Demographic data of the participants

	Mixed grain ($N = 14$)	Regular diet ($N = 14$)	*P* value
Age (y)	16.21 ± 0.43	16.21 ± 0.70	1.000
Education (y)	9.57 ± 0.51	9.57 ± 0.51	1.000
Height (cm)	174.67 ± 5.34	176.49 ± 5.29	0.151
Weight (kg)	64.48 ± 7.72	69.31 ± 11.40	0.964
Body mass index	21.09 ± 1.98	22.20 ± 3.34	0.302

Table 4
Results of vital signs and laboratory tests

	Mixed-grain ($N = 14$)			Regular diet ($N = 14$)			P value[1]	P value[2]	P value[3]	P value[4]
	Baseline	Endpoint	Change*	Baseline	Endpoint	Change*				
Systolic blood pressure (mmHg)	123.79 ± 12.72	134.07 ± 10.59†	10.29 ± 15.66	119.79 ± 11.06	130.79 ± 13.34†	11.00 ± 9.00	.056	.884	.350	.000
Diastolic blood pressure (mmHg)	69.07 ± 4.14	78.00 ± 8.67†	8.93 ± 7.10	70.36 ± 5.54	79.00 ± 7.01†	8.64 ± 7.33	.908	.917	.586	.000
Pulse rate (bpm)	75.93 ± 12.41	78.50 ± 15.76	2.57 ± 12.54	76.00 ± 12.11	79.57 ± 10.64	3.57 ± 10.29	.487	.819	.897	.168
Temperature (°C)	36.81 ± 0.31	36.24 ± 0.68†	−0.56 ± 0.66	36.69 ± 0.26	36.31 ± 0.52†	−0.37 ± 0.51	.369	.393	.860	.000
WBC ($\times 10^3$ cells/μL)	6.66 ± 1.20 (0/14)	6.61 ± 1.64 (1/14)	−0.05 ± 1.53	7.13 ± 1.19 (0/14)	6.65 ± 1.84 (1/14)	−0.47 ± 0.93	.082	.387	.628	.279
RBC ($\times 100^3$ cells/μL)	5.30 ± 0.29 (0/14)	5.05 ± 0.48 (1/14)	−0.25 ± 0.54	5.23 ± 0.24 (0/14)	5.13 ± 0.17† (0/14)	−0.10 ± 0.16	.000	.320	.994	.029
Hemoglobin (g/dL)	15.64 ± 0.85 (0/14)	15.31 ± 0.81† (0/14)	−0.33 ± 0.31	15.68 ± 0.65 (0/14)	15.42 ± 0.69 (0/14)	−0.26 ± 0.49	.130	.648	.778	.000
Hematocrit (%)	46.62 ± 2.43 (0/14)	45.49 ± 2.37 (0/14)	−1.13 ± 2.02	46.99 ± 1.78 (0/14)	46.45 ± 1.80 (0/14)	−0.54 ± 1.58	.386	.271	.209	.158
Platelet ($\times 10^3$ cells/μL)	238.79 ± 62.56 (1/14)	248.64 ± 47.90 (0/14)	9.86 ± 34.88	254.14 ± 50.42 (0/14)	257.29 ± 49.38 (0/14)	3.14 ± 31.01	.678	.595	.533	.307
AST (IU/L)	17.93 ± 2.30 (0/14)	23.86 ± 3.35† (0/14)	5.93 ± 4.20	23.07 ± 21.28 (1/14)	22.79 ± 5.29 (1/14)	−0.29 ± 18.45	.000	.230	.551	.275
ALT (IU/L)	13.93 ± 4.34 (0/14)	17.21 ± 5.37†(0/14)	3.29 ± 2.79	17.00 ± 7.49 (0/14)	19.71 ± 9.67 (1/14)	2.71 ± 7.21	.002	.784	.265	.007
Total protein (g/dL)	7.75 ± 0.47 (2/14)	8.09 ± 0.42† (4/14)	0.34 ± 0.43	7.56 ± 0.67 (2/14)	8.07 ± 0.40† (4/14)	0.51 ± 0.72	.070	.451	.491	.000
Albumin (g/dL)	4.77 ± 0.21 (0/14)	4.84 ± 0.17 (0/14)	0.07 ± 0.25	4.66 ± 0.28 (0/14)	4.89 ± 0.19† (0/14)	0.23 ± 0.38	.156	.181	.624	.013
BUN (mg/dL)	12.86 ± 1.92 (0/14)	14.57 ± 2.53† (0/14)	1.71 ± 2.46	13.00 ± 2.00 (0/14)	13.71 ± 3.29 (0/14)	0.71 ± 3.36	.275	.377	.643	.038
Creatinine (mg/dL)	0.73 ± 0.11 (6/14)	0.82 ± 0.09† (1/14)	0.09 ± 0.09	0.72 ± 0.07 (8/14)	0.81 ± 0.08† (2/14)	0.09 ± 0.07	.367	.909	.772	.000
Cholesterol (mg/dL)	145.00 ± 23.55 (0/14)	140.57 ± 17.93 (0/14)	−4.43 ± 12.50	138.50 ± 24.21 (0/14)	143.43 ± 18.98 (0/14)	4.93 ± 16.60	.319	.104	.812	.929
Triglycerides (mg/dL)	95.64 ± 42.15 (1/14)	87.86 ± 24.37 (0/14)	−7.79 ± 38.79	97.50 ± 75.22 (1/14)	85.64 ± 43.36 (0/14)	−11.86 ± 62.81	.094	.838	.991	.329

P value[1], t-test between changes of the mixed-grain and regular diet; P value[2,3,4], interaction, group effect, and time effect of two-way repeated measure ANOVA, respectively
ALT, alanine transaminase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; RBC, red blood cell; WBC, white blood cell

* Change = Endpoint–baseline.

† Paired t-test between baseline and endpoint ($P < .05$).

improvements in comprehension and memory in the regular-diet group. However, no significant differences between the change scores of the two groups were observed. For the SACL (Table 7), a significant increase in the total arousal score was demonstrated in the regular-diet group, but no significant differences between the two groups were found. Plasma BDNF and S100B levels (Table 8) showed no change between the baseline and endpoint in either group, but a significant difference between the two groups was observed in changes in BDNF levels.

Discussion

We sought to establish whether consumption of a mixed-grain or regular diet influences cognitive performance and plasma BDNF and S100B levels in healthy high school students. The results showed a positive effect of mixed grains on a mental-fatigue test and on plasma BDNF levels.

In the CNT 40, significant improvements in VLT and TMT B at the endpoint were observed in both the mixed-grain and the regular-diet groups, but no significant differences in effects between the two groups were observed in most change scores. These results suggest that the mixed-grain diet is not superior with respect to its effects on neuropsychological tests compared with the regular diet. It seems that the improvements in some areas of cognitive performance in both groups may have been related to practice effects resulting from repetition of the same tests rather than representing true improvements in cognitive functioning. It has been reported that practice effects may appear even after 6 [26] or 12 mo [27], and tests requiring complex cognitive processing and those where the formulation of a strategy may aid performance show greater practice effects than do tests such as the WCST and the Stroop test, which measure more simple cognitive functions [27]. To avoid this methodological shortcoming, it is recommended that researchers use alternate forms or select tests with less pronounced practice effects [28], and this issue should be considered in designing future studies.

In the mental-fatigue tests, no significant changes were observed in the endpoint scores of the mixed-grain group compared with baseline. However, a significant deterioration in scores in the AX-CPT was noted at the endpoint in the regular-diet group, and comparisons of the change scores for the AX-CPT between the groups revealed significant differences. The impairment in the AX-CPT scores in the regular-diet group might have been caused by direct harmful effects of the regular diet, which seems unlikely because it was a normal healthy diet, or might be derived from some other external factors. The latter may have been related to mental stress resulting from high academic pressure and frequent examinations. This hypothesis can be supported by the significant increase in the total arousal score on the SACL at the endpoint compared with baseline in the regular-diet group. Interestingly, there were no significant changes in the SACL from baseline to endpoint in the mixed-grain group. Hence, these findings suggest that our hypothesis that mixed grains may have some protective effects against mental stress was confirmed in the case of a highly demanding cognitive task, i.e., the mental-fatigue test. Several mechanisms underpinning these protective effects of mixed grains on mental-fatigue need to be considered. First, it may be related to their relatively high concentrations of some ingredients, such as B vitamins, minerals, and GABA, compared with the regular diet. Among these, GABA is of particular interest because of its inhibitory role in the central nervous system. GABA has been implicated in many diseases such as epilepsy, mood disorders, schizophrenia, and dementia [29,30]. Studies suggest beneficial

Table 5
Results of CNT 40 in the mixed-grain and regular diet groups

	Mixed–grain ($N = 14$)			Regular diet (N = 12)			P value[1]	P value[2]	P value[3]	P value[4]
	Baseline	Endpoint	Change*	Baseline	Endpoint	Change*				
Attention										
Digit span test										
Forward	7.64 ± 0.63	7.71 ± 0.61	0.07 ± 0.47	7.75 ± 0.45	7.92 ± 0.29	0.17 ± 0.39	.585	.746	.295	.419
Backward	5.79 ± 1.37	6.29 ± 1.07	0.50 ± 1.09	6.08 ± 1.08	6.50 ± 0.80	0.42 ± 1.24	.857	.893	.411	.144
Auditory CPT										
Correct response	131.93 ± 3.43	132.50 ± 3.80	0.57 ± 2.03	131.50 ± 4.30	132.33 ± 3.11	0.83 ± 2.79	.784	.899	.773	.496
Incorrect response	3.86 ± 4.17	2.93 ± 3.83	−0.93 ± 4.95	6.58 ± 7.13	5.42 ± 6.17	−1.17 ± 6.64	.918	.937	.088	.488
Sensitivity index	0.99 ± 0.01	0.99 ± 0.01	0.00 ± 0.01	0.99 ± 0.01	0.99 ± 0.01	0.00 ± 0.01	.538	.845	.295	.371
Stroop test										
Color	13.91 ± 2.56	13.67 ± 2.63	−0.24 ± 1.99	13.16 ± 2.49	13.27 ± 1.98	0.11 ± 1.74	.643	.799	.402	.921
Word–color	19.91 ± 2.91	19.45 ± 3.48	−0.45 ± 2.59	18.37 ± 2.42	18.34 ± 4.25	−0.03 ± 2.33	.666	.819	.158	.795
Interference score	6.00 ± 3.12	5.79 ± 2.93	−0.21 ± 2.26	5.21 ± 2.55	5.07 ± 3.39	−0.14 ± 2.57	.935	.963	.373	.836
Memory										
Verbal learning test										
A1	6.93 ± 1.73	10.93 ± 2.97†	4.00 ± 3.23	8.08 ± 2.50	10.75 ± 2.18†	2.67 ± 2.81	.277	.322	.467	.000
A5	14.21 ± 0.97	14.50 ± 0.85	0.29 ± 0.83	13.83 ± 1.47	14.58 ± 0.67	0.75 ± 1.42	.310	.420	.604	.076
Delayed recall at 20 min	13.14 ± 2.18	13.93 ± 1.00	0.79 ± 1.72	12.08 ± 2.50	14.25 ± 1.29†	2.17 ± 1.64	.048	.183	.473	.006
Try A1 ~ A5 total	56.29 ± 9.59	67.21 ± 6.80†	10.93 ± 7.05	58.33 ± 10.09	66.33 ± 6.73†	8.00 ± 8.71	.353	.536	.805	.000
Executive functioning										
Trail making test B	27.36 ± 6.20	24.07 ± 5.72†	−3.29 ± 5.03	29.33 ± 8.71	25.17 ± 11.28†	−4.17 ± 4.86	.655	.846	.499	.105
Wisconsin card sorting test										
Category completed	6.00 ± 0.00	5.93 ± 0.27	−0.07 ± 0.27	6.00 ± 0.00	5.92 ± 0.29	−0.08 ± 0.29	.914	.914	.914	.162
Perseverative response	11.71 ± 5.18	9.14 ± 3.90	−2.57 ± 6.16	9.92 ± 5.74	9.42 ± 3.20	−0.50 ± 7.66	.452	.424	.556	.237
Perseverative error	8.36 ± 3.05	6.50 ± 1.91	−1.86 ± 3.44	7.08 ± 3.18	7.08 ± 3.18	−0.17 ± 3.61	.234	.226	.537	.148
Trials to complete first category	12.00 ± 3.42	13.64 ± 5.85	1.64 ± 6.70	12.75 ± 3.31	12.42 ± 3.23	−0.33 ± 4.33	.390	.398	.838	.575
Language										
Word-fluency test										
Animal	22.64 ± 2.92	24.57 ± 3.65	1.93 ± 4.16	21.08 ± 2.64	21.92 ± 4.06	0.83 ± 4.15	.509	.561	.029	.146
Stationery	23.29 ± 4.94	23.43 ± 5.75	0.14 ± 4.47	18.58 ± 3.92	21.50 ± 6.90	2.92 ± 4.76	.139	.367	.034	.320
"ㄱ"	16.50 ± 4.42	19.71 ± 5.76	3.21 ± 6.12	15.33 ± 3.73	17.33 ± 3.94	2.00 ± 3.74	.556	.636	.170	.046
"ㅅ"	15.57 ± 5.32	18.64 ± 5.17	3.07 ± 7.33	15.42 ± 2.50	16.58 ± 3.82	1.17 ± 3.64	.422	.444	.374	.092
"ㅇ"	14.79 ± 4.39	16.71 ± 4.48	1.93 ± 6.40	15.50 ± 4.70	16.75 ± 4.00	1.25 ± 3.28	.732	.783	.761	.201
Visuomotor speed										
Trail making test A	20.71 ± 7.15	7.15 ± 2.73	−2.36 ± 6.26	20.08 ± 4.93	19.33 ± 5.28	−0.75 ± 5.79	.506	.586	.907	.295

P value[1], t-test between changes of the mixed-grain and regular diet; P value[2,3,4], interaction, group effect, and time effect of two-way repeated measure ANOVA, respectively
CPT, continuous performance test
* Change = Endpoint – baseline.
† Paired t-test between baseline and endpoint ($P < .05$).

Table 6
Results of mental fatigue tests in the mixed-grain and regular diet groups

	Mixed–grain ($N = 14$)			Regular diet ($N = 12$)			P value[1]	P value[2]	P value[3]	P value[4]
	Baseline	Endpoint	Change*	Baseline	Endpoint	Change*				
First RVIP										
Correct response	36.64 ± 5.61	37.57 ± 2.53	0.93 ± 5.99	33.33 ± 8.67	34.83 ± 9.64	1.50 ± 9.13	.850	.884	.126	.535
Reaction time (ms)	468.91 ± 100.53	431.26 ± 66.68	−37.66 ± 65.74	475.87 ± 74.61	453.82 ± 95.36	−22.05 ± 62.60	.543	.774	.538	.215
Incorrect response	8.07 ± 22.50	1.71 ± 1.49	−6.36 ± 23.00	11.50 ± 27.50	8.75 ± 28.12	−2.75 ± 7.05	.607	.771	.401	.464
AX-CPT										
Target trial										
Correct response	414.00 ± 5.92	413.86 ± 5.72	−0.14 ± 7.21	411.42 ± 10.62	402.58 ± 20.35†	−8.83 ± 12.59	.038	.192	.040	.178
Incorrect response	11.57 ± 13.37	9.43 ± 10.71	−2.14 ± 8.81	10.67 ± 15.54	11.00 ± 7.64	0.33± 13.13	.573	.716	.922	.790
Omission error	6.00 ± 5.92	6.14 ± 5.72	0.14 ± 7.21	8.58 ± 10.62	17.42 ± 20.35†	8.83 ± 12.59	.038	.192	.040	.178
Nontarget trial										
Correct response	176.79 ± 2.12	177.50 ± 2.71	0.71 ± 2.79	174.92 ± 5.74	174.25 ± 5.55	−0.67 ± 5.03	.386	.559	.034	.984
Incorrect response	6.79 ± 4.93	4.29 ± 3.38	−2.50 ± 4.88	9.00 ± 10.69	9.50 ± 13.48	0.50 ± 6.39	.188	.543	.136	.685
Omission error	3.21 ± 2.12	2.50 ± 2.71	−0.71 ± 2.79	7.83 ± 10.55	4.92 ± 5.26	−2.92 ± 9.97	.435	.507	.038	.276
Sensitivity index	0.98 ± 0.02	0.98 ± 0.02	0.00 ± 0.02	0.98 ± 0.03	0.97 ± 0.02	−0.01 ± 0.02	.262	.448	.502	.818
Second RVIP										
Correct response	36.64 ± 4.05	37.79 ± 2.46	1.14 ± 4.62	32.50 ± 9.97	34.17 ± 8.22	1.67 ± 9.48	.856	.888	.041	.452
Reaction time (ms)	443.26 ± 67.07	422.21 ± 56.26	−21.04 ± 51.27	462.38 ± 85.61	466.55 ± 75.18	4.18 ± 48.87	.214	.527	.115	.672
Incorrect response	2.14 ± 2.28	1.57 ± 1.79	−0.57 ± 2.28	22.50 ± 49.99	9.08 ± 26.14	−13.42 ± 45.27	.347	.398	.070	.357

AX-CPT, AX-continuous performance test; RVIP, rapid visual information processing
P value[1], t-test between changes of the mixed-grain and regular diet; P value[2,3,4], interaction, group effect, and time effect of two-way repeated measure ANOVA, respectively
* Change = Endpoint – baseline.
† Paired t-test between baseline and endpoint ($P < .05$).

Table 7
Results of self-rating scales for cognitive functioning and SACL

	Mixed–grain ($N = 14$)			Regular diet ($N = 12$)			P value[1]	P value[2]	P value[3]	P value[4]
	Baseline	Endpoint	Change*	Baseline	Endpoint	Change*				
Cognitive functioning										
Concentration	3.86 ± 1.23	4.64 ± 0.93	0.79 ± 1.76	3.17 ± 0.58	4.17 ± 1.59	1.00 ± 1.65	.753	.737	.072	.007
Comprehension	4.14 ± 0.66	4.57 ± 0.65	0.43 ± 0.76	3.67 ± 0.78	4.75 ± 1.29†	1.08 ± 1.51	.165	.181	.540	.003
Memory	4.29 ± 0.47	4.57 ± 1.09	0.29 ± 1.20	3.75 ± 1.06	4.75 ± 1.29†	1.00 ± 1.54	.197	.209	.527	.026
Verbal expression	4.07 ± 0.73	4.64 ± 0.84	0.57 ± 1.02	4.75 ± 0.62	4.75 ± 1.14	0.00 ± 1.35	.230	.233	.103	.233
Executive function	3.71 ± 0.73	4.50 ± 0.52†	0.79 ± 0.89	4.17 ± 0.94	4.75 ± 1.06	0.58 ± 1.83	.717	.659	.130	.004
Persistence	4.00 ± 1.41	3.93 ± 0.83	−0.07 ± 1.27	3.83 ± 1.75	4.58 ± 1.38	0.75 ± 2.34	.267	.285	.524	.376
Task processing speed	3.79 ± 0.43	4.43 ± 0.94†	0.64 ± 0.93	4.25 ± 0.75	4.67 ± 1.23	0.42 ± 1.16	.587	.644	.155	.034
Total	27.86 ± 3.23	31.29 ± 2.67†	3.43 ± 4.03	27.58 ± 3.58	32.42 ± 6.83	4.83 ± 8.05	.571	.559	.721	.001
SACL										
Total stress	35.93 ± 9.08	35.86 ± 8.41	−0.07 ± 10.08	36.50 ± 7.33	31.50 ± 5.70	−5.00 ± 9.91	.222	.263	.389	.250
Total arousal	34.29 ± 4.91	34.07 ± 3.99	−0.21 ± 4.41	30.50 ± 5.13	33.50 ± 4.95†	3.00 ± 4.61	.082	.229	.105	.296
Total	70.21 ± 11.09	69.93 ± 10.17	−0.29 ± 10.92	67.00 ± 9.45	65.00 ± 5.98	−2.00 ± 8.97	.669	.747	.129	.667

SACL, stress-arousal checklist
P value[1], t-test between changes of the mixed-grain and regular diet; P value[2,3,4], interaction, group effect, and time effect of two-way repeated measure ANOVA, respectively
* Change = Endpoint – baseline.
† Paired t-test between baseline and endpoint ($P < .05$).

effects of GABA in lowering hypertension [13], inhibiting cancer-cell proliferation [15], and enhancing memory performance [16]. Because of these health-promoting effects of GABA, many functional foods with a high GABA content have been developed. The presumed protective effects of mixed grains may be consistent with the results of Kim and Yoon [31], who found that drinking green tea containing GABA increased α and θ waves. Taken together, these findings lead us to speculate that mixed grains with a high GABA content may exert some calming effect, and this may have resulted in no change in the total arousal score at endpoint and a beneficial effect in the mental-fatigue test. B vitamins may affect cognitive function through their roles in neurotransmitter synthesis and modulation, axon and myelin sheath integrity, and homocysteine metabolism [32]. Although there is greater interest in the role of vitamins in the cognitive development of children and in the maintenance of cognitive performance in elderly people, few studies have been carried out in adolescents or younger adults. Several studies have reported some improvement in mental performance [33], concentration [34,35], or stress-related parameters [36,37] after treatment with multivitamin–mineral supplementation. As for minerals, except for iodine and iron, there is limited information available regarding the effect of other micronutrients on cognitive function. A few studies have been conducted to investigate the role of calcium in old age [38] and of magnesium in patients undergoing preterm labor [39]. Considering the greater ratio of B vitamins and some minerals in a mixed-grain compared with a regular diet, it can be speculated that greater supplementation of vitamins and minerals may have prevented cognitive deterioration in the mixed-grain group. However, as we did not measure serum levels of vitamins and minerals, this interpretation should remain highly speculative. Second, as the mixed-grain diet is likely to have a lower glycemic index than the regular diet due to the higher protein and fiber content, it would provide more stable blood glucose levels, which have also been implicated in improved cognitive capacity children and adolescents [40]. Additionally, the lower glycemic index and higher protein and fiber content of the mixed grain diet compared with the regular diet may have increased feelings of satiety [41] in the intervention group, which may have improved cognitive performance by reducing feelings of hunger.

BDNF is a member of the nerve-growth factor family, which supports differentiation and survival of diverse populations of neurons in the peripheral and central nervous systems during development. There is evidence that acute and chronic stress decrease the expression of BDNF in the hippocampus [42]. Therefore, it was interesting to observe significant differences in the changes in BDNF levels in the two groups. It may be that mental stress during the course of the study contributed to the reduced BDNF levels in the regular-diet group, whereas mixed grains prevented this stress-induced decrease in BDNF. However, as the degree of change in BDNF levels in the regular-diet group was relatively small, the significance of the BDNF findings needs to be readdressed in a future study with a larger sample size.

The strength of the present study was the use of a mental-fatigue test, which provides a different model for demonstrating effects on cognitive function, i.e., evaluating cognitive deterioration, not cognitive enhancement. This approach may be useful in a study targeting relatively well-nourished subjects under considerable psychological stress. In addition, to the best of our knowledge, this is the first report investigating a change on the possible biological markers, BDNF and S100B, after diet

Table 8
Results of plasma BDNF (pg/mL) and S100B (μg/L) levels in the mixed-grain and regular diet groups

	Mixed–grain ($N = 14$)			Regular diet ($N = 14$)			P value[1]	P value[2]	P value[3]	P value[4]
	Baseline	Endpoint	Change*	Baseline	Endpoint	Change*				
BDNF	987.39 ± 0.43	987.59 ± 0.33	0.21 ± 0.46	987.66 ± 0.40	987.12 ± 1.13	−0.54 ± 1.10	.030	.135	.569	.341
S100B	0.04 ± 0.02	0.05 ± 0.03	0.01 ± 0.04	0.03 ± 0.02	0.05 ± 0.03	0.01 ± 0.02	.952	.959	.226	.113

BDNF, brain-derived neurotrophic factor
P value[1], t-test between changes of the mixed-grain and regular diet; P value[2,3,4], interaction, group effect, and time effect of two-way repeated measure ANOVA, respectively
* Change = Endpoint – baseline.

intervention. Several limitations of the study should be noted. The first is the appropriateness of the cognitive tests that were selected for use. In a review by Hoyland et al. [43], memory tasks and other tasks that required greater cognitive load were identified as the cognitive modality most sensitive to macronutrient manipulations. For young, intelligent participants, selecting tasks requiring greater cognitive load is regarded as being more important. As our objective was to evaluate the effects of nutritional intervention on diverse domains of cognitive function, we did not select cognitive tests based on the task sensitivity. The mental-fatigue test can be viewed as requiring a substantial amount of cognitive effort. The second limitation is the small sample size and the non-representative nature of the sample. Because of the scarcity of literature on the effect of nutritional interventions in adolescents and the heterogeneity of the cognitive tests employed, we did not perform sample size and power calculation. As the mean change scores in the present study were small, with relatively large standard deviations, increasing the sample size would lower the probability of a type II error (false negative). As all of the enrolled subjects were living in a dormitory where only students with good grades can be admitted, the generalizability of our results is limited. The third limitation relates to the timing of the administration of the cognitive tests, which was from 8 pm to 10 p.m., whereas most previous studies have selected the morning for testing. The reason for choosing the evening was that the school did not allow any assessments during the regular school hours (9 am to 6 pm). Even though evidence suggests that effects of nutritional intervention, especially glucose study, are more clearly demonstrable in the morning [41,44], no study has addressed the advantages and disadvantages of test timing between morning and evening. However, selection of test timing should be carefully considered in future research. The fourth limitation is the sufficiency of the intervention period. We had originally set the intervention period at 8 wk. However, because of the sudden scheduling of an examination just before the endpoint, this was extended to 9 wk. Although intervention periods are quite varied, from 2 wk to several years depending on the objectives of the studies, many have adopted 8 wk [45–47], and we intended to use a comparable length of time. Given that the participants in the study were young, healthy, and well nourished, 9 wk seems rather short. Therefore, it may be that the lack of effect observed on most cognitive measures could be attributed to an insufficient intervention period for nutritional manipulation to take effect. Finally, we did not measure the IQs of participants, and this may have affected the results. However, it is not likely given that baseline scores for the CNT and mental-fatigue test were no different between the two groups (data not shown) and that all the participants had generally good grades.

In conclusion, our findings suggest that intake of mixed grains for 9 wk has beneficial effects on cognitive performance and plasma BDNF levels in high school students. These beneficial effects seem related to the prevention of cognitive deterioration in a mental-fatigue test when on a mixed-grain diet rather than to cognitive enhancement per se. Also, effects may be related to the high content of vitamins, minerals, and GABA in the mixed-grain product. Future studies are needed to confirm these findings with a larger sample size.

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References

[1] Bellisle F. Effects of diet on behavior and cognition in children. Br J Nutrition 2004;92(suppl 2):S227–32.
[2] Benton D. The influence of dietary status on the cognitive performance of children. Mol Nutr Food Res 2010;54:457–70.
[3] Taras H. Nutrition and student performance at school. J Sch Health 2005;75:199–213.
[4] Gogtay N, Giedd JN, Lusk L, Hayashi KM, Greenstein D, Vaituzis AC, et al. Dynamic mapping of human cortical development during childhood through early adulthood. Proc Natl Acad Sci USA 2004;101:8174–9.
[5] Giedd JN, Blumenthal J, Jeffries NO, Castellanos FX, Liu H, Zijdenbos A, et al. Brain development during childhood and adolescence: a longitudinal MRI study. Nat Neurosci 1999;2:861–3.
[6] Chiron C, Raynaud C, Mazière B, Zilbovicus M, Laflamme L, Masure M-C, et al. Changes in regional cerebral blood flow during brain maturation in children and adolescents. J Nucl Med 1992;33:696–703.
[7] Paus T, Zijdenbos A, Worsley K, Collins DL, Blumenthal J, Giedd JN, et al. Structural maturation of neural pathways in children and adolescents: an in vivo study. Science 1999;283:1908–11.
[8] Steinberg L. Cognitive and affective development in adolescence. Trends Cogn Sci 2005;9:69–74.
[9] Ivanovic D, Vasquez M, Aguayo M, Ballester D, Marambio M, Zacarias I. Nutrition and education. III. Educational achievement and food habits of Chilean elementary and high school graduates. Arch Latinoam Nutr 1992;4291:9–14.
[10] Alaimo K, Olson CM, Frongillo EA Jr. Food insufficiency and American school-aged children's cognitive, academic, and psychosocial development. Pediatrics 2001;108:44–53.
[11] Abalkhail B, Shawky S. Prevalence of daily breakfast intake, iron deficiency anaemia, and awareness of being anaemic among Saudi school students. Int J Food Sci Nutr 2002;53:519–28.
[12] Shu XL, Frank T, Shu QY, Engel KH. Metabolite profiling of germinating rice seeds. J Agric Food Chem 2008;56:11612–20.
[13] Yoshimura M, Toyoshi T, Sano A, Izumi T, Fujii T, Konishi C, et al. Antihypertensive effect of a γ-aminobutyric acid rich tomato cultivar “DG03-9” in spontaneously hypertensive rats. J Agric Food Chem 2010;58:615–9.
[14] Oh SH, Soh JR, Cha YS. Germinated brown rice extract shows a nutraceutical effect in the recovery of chronic alcohol-related symptoms. J Med Food 2003;6:115–21.
[15] Oh CH, Oh SH. Effect of germinated brown rice extracts with enhanced levels of GABA on cancer cell proliferation and apoptosis. J Med Food 2004;7:19–23.
[16] Chambers MS, Atack JR, Carling RW, Collinson N, Cook SM, Dawson GR, et al. An orally bioavailable, functionally selective inverse agonist at the benzodiazepine site of $GABA_A$ α_5 receptors with cognition enhancing properties. J Med Chem 2004;47:5829–32.
[17] Nutt DJ, Besson M, Wilson SJ, Dawson GR, Lingford-Hughes R. Blockade of alcohol's amnestic activity in humans by an α_5 subtype benzodiazepine receptor inverse agonist. Neuropharmacology 2007;53:810–20.
[18] Han OS, Hong JP. Structured clinical interview for DSM-IV axis I disorders, Seoul, Hana Medical Publisher: Research version; 2000.
[19] First M, Spitzer RL, Gibbon M, William JB. Structured Clinical Interview for DSM-IV Axis I Disorders, Research Version, Non-patient ed. New York: Biometrics Research, New York State Psychiatric Institute; 1997.
[20] AOAC. Official Methods of Analysis of AOAC Int1. 13th ed. Methods 930.04, 930.5, 979.09 and 957.13. Washington, DC: Association of Official Analytical Chemists; 1990.
[21] Moore S, Spackman DH, Stein WH. Automatic recording apparatus for use in the chromatography of amino acids. Fed Proc 1958;17:1107–15.
[22] KFDA. Korea Food and Drug Administration. Food Code. Seoul, Korea: Korean Foods Industry Association; 2005a. p. 368–90.
[23] Marszall ML, Lcbicdzińska A, Czamowski W, Szcfer P. High-performance liquid chromatography method for the simultaneous determination of hydrochloride, pyridoxine hydrochloride and cyanocobalamin in pharmaceutical formulations using coulometric electrochemical and ultraviolet detection. J Chromatogr A 2005;1094:91–8.
[24] KFDA. Korea Food and Drug Administration. Food Code. Seoul, Korea: Korean Foods Industry Association; 2005b. p. 358–364.
[25] Mackay C, Cox T, Burrows G, Lazzerini T. An inventory for the measurement of self-reported stress and arousal. Br J Soc Clin Psychol 1978;17:283–4.
[26] Basso MR, Carona FD, Lowery N, Axelrod BN. Practice effects on the WAIS-III across 3- and 6-month intervals. Clin Neuropsychol 2002;16:57–63.
[27] Basso MR, Bornstein RA, Lang JM. Practice effects on commonly used measures of executive function across twelve months. Clin Neuropsychol 1999;13:283–92.
[28] Dikmen SS, Heaton RK, Grant I, Temkin NR. Test–retest reliability and practice effects of expanded Halstead–Reitan neuropsychological test battery. J Int Neuropsychol Soc 1999;5:346–56.

[29] Bernard C, Cossart R, Hirsch JC, Esclapes M, Ben-Ari Y. What is GABAergic inhibition? How is it modified in epilepsy? Epilepsia 2000;41:S90–5.
[30] Arrúe A, Dávila R, Zumárraga M, Basterreche N, González-Torres MA, Goienetxea B, et al. GABA and homovanillic acid in the plasma of schizophrenic and bipolar I patients. Neurochem Res 2010;35:247–53.
[31] Kim SI, Yoon SW. Analytical study for the effects of green tea with GABA about the brain wave and short-term memory. J Korean Jungshin Sci Soc 1998;2:84–91.
[32] Huskisson E, Maggini S, Ruf M. The influence of micronutrients on cognitive function and performance. J Int Med Res 2007;35:1–19.
[33] Heseker H, Kűbler W, Pudel V, Westenhofer J. Interaction of vitamins with mental performance. Bibl Nutr Dieta 1995;52:43–55.
[34] Carroll D, Ring C, Suter M, Willemsen G. The effects of an oral multivitamin combination with calcium, magnesium and zinc on psychological well-being in healthy young male volunteers: a double-blind placebo-controlled trial. Psychopharmacology 2000;150:220–5.
[35] Popovic IC. Neurotrope vitamin-minerals toff kombination in der stress-therapie. Schweiz Zeitschr Ganzh Med 1993;3:140–3.
[36] Gruenwald J, Graubaum HJ, Harde A. Effect of a probiotic multivitamin compound on stress and exhaustion. Adv Ther 2002;19:141–50.
[37] Schlebusch L, Bosch BA, Polglase G, Kleinschmidt I, Pillay BJ, Cassimjee MH. A double-blind, placebo-controlled, double-centre study of the effects of an oral multivitamin-mineral combination on stress. S Afr Med J 2000;90:1216–23.
[38] Schram MT, Trompet S, Kamper AM, de Craen AJM, Hofman A, Euser SM, et al. Serum calcium and cognitive function in old age. J Am Geriatr Soc 2007;55:1786–92.
[39] Ghia N, Spong CY, Starbuck VN, Scialli AR, Ghidini A. Magnesium sulfate therapy affects attention and working memory in patients undergoing preterm labor. Am J Obstet Gynecol 2000;183:940–4.
[40] Micha R, Rogers PJ, Nelson M. The glycaemic potency of breakfast and cognitive function in school children. Eur J Clin Nutr 2010;64:948–57.
[41] Dye L, Blundell J. Functional foods: psychological and behavioral functions. Br J Nutr 2002;88(suppl 2):S187–211.
[42] Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. Biol Psychiatry 2006;59:1116–27.
[43] Hoyland A, Lawton CL, Dye L. Acute effects of macronutrient manipulations on cognitive test performance in healthy young adults: a systematic research review. Neurosci Biobehav Rev 2008;32:72–85.
[44] Messier C. Glucose improvement of memory: a review. Eur J Pharmacol 2004;490:33–57.
[45] Bruner AB, Joffe A, Duggan AK, Casella JF, Brandt J. Randomized study of cognitive effects of iron supplementation in non-anaemic, iron-deficient adolescent girls. Lancet 1996;348:1789–92.
[46] Fioravanti M, Ferrario E, Massaia M, Cappa G, Rivolta G, Grossi E, et al. Low folate levels in the cognitive decline of elderly patients and efficacy of folate as a treatment for improving memory deficits. Arch Gerontol Geriat 1997;26:1–13.
[47] Passeri M, Cucinotta D, Abate G, Senin U, Ventura A. Oral 5′-methyltetrahydrofolic acid in senile organic mental disorders with depression: results of a double-blind multicenter study. Aging Clin Exp Res 1993;5:63–71.