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Effects of Brain-directed Nutrients on Cerebral Blood Flow and Neuropsychological Testing: A Randomized, Double-blind, Placebo-controlled, Crossover Trial

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ABSTRACT

Context • In a prior open trial of professional football players who displayed the effects of traumatic brain injury, the current reserach team reported significant improvements in clinical symptoms, neuropsychological testing and regional cerebral blood flow (rCBF) following the use of brain-directed nutrients (BDNs) and lifestyle interventions.

Objective • The current study intended to determine whether supplementation with BDNs improved rCBF and neuropsychological function in healthy individuals.

Design • The current study was a randomized, double-blind, placebo-controlled, crossover trial, which was a more rigorous reseach design than the prior study and did not include lifestyle interventions.

Setting • Participants underwent evaluation and testing at the Amen Clinics, Inc, a private medical facility in Newport Beach, CA.

Participants • Thirty healthy adult (15 male and 15 female) participants were recruited from the community though local advertising and met the requirements for eligibility into the study. Twenty-five individuals completed the study, with dropout due to events unrelated to the study itself.

Intervention • The participants were randomly assigned to a treatment order for intervention, either placebo or brain supplements first. The BDNs treatment was comprised of three supplements: fish oil; a high-potency, multiple vitamin/mineral supplement; and a brain-enhancement supplement. The placebo treatment was two supplements comprised of rice flour to replace the multiple vitamin/mineral complex and the brain-enhancement supplement and one supplement made of other oils to replace the fish-oil mixture. After 2 mo of this first intervention, a crossover intervention occurred for a final 2 mo, in which participants formerly receiving BDNs received a placebo treatment and participants formerly treated with placebo received the BDNs treatment.

Outcome Measures • Primary outcome measures included (1) an analysis of the changes in rCBF using SPECT and (2) an assessment of the differences in cognitive and emotional function using the MicroCog (cognitive performance), the WebNeuro (emotional state), and three psychological inventories—the Beck Depression Inventory (BDI-II), Brief Symptom Inventory (BSI), and Quality of Life Inventory (QOLI).

Results • A region of interest (ROI) analysis for each of the 2-mo phases (baseline, then placebo and treatment according to randomized order) showed significant improvement in rCBF for the BDNs as compared to the placebo (as assigned at the start of the first intervention) in the prefrontal cortex, anterior and posterior cingulate gyrus, hippocampus, and cerebellum. Significant improvements were observed for the BDNs (1) on the MicroCog—reasoning, $P = .008$; memory, $P = .014$; information processing accuracy, $P = .027$; (2) on the WebNeuro—executive function, $P = .002$, information processing efficiency, $P = .015$; depressed mood, $P = .017$, and emotional identification, $P = .041$; and (3) on the BSI—positive symptom total, $P = .024$ and reduced hostility, $P = .018$. For the last, significance occurred upon accounting for the effect of order.

Conclusion • This study demonstrates the potential effectiveness of BDNs in enhancing rCBF and neuropsychological function across various cognitive and psychological domains. (*Adv Mind Body Med.* 2013;27(2):24-33.)

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The use of natural, plant-based supplements and Chinese herbs to support cognitive health and reduce brain aging is an evolving field, with nutrients being more frequently investigated as a first-line therapeutic strategy in clinical settings. Evidence in the literature supports the use of nutrient-based therapies for this purpose, but more high-quality, double-blind, randomized, controlled trials (RCTs) are required to explore their efficacy. It has been shown that older individuals who are cognitively normal may have undiagnosed Alzheimer's disease (AD) pathology[1] and cerebrovascular disease.[2] Several herbal ingredients have been shown to be clinically effective at improving brain function by enhancing antioxidant, cholinergic, and cerebrovascular function.

One such ingredient is huperzine A, an alkaloid extracted from Chinese club moss, *Huperzia serrata*. It functions as a reversible, selective inhibitor of acetylcholinesterase[3] but has also been shown to protect against oxidative injury, neuronal apoptosis, and glutamate-induced toxicity.[4,5] *Ginkgo biloba* is a Chinese herb that has been shown to improve vascular function, scavenge free radicals, lower oxidative stress, and protect mitochondrial function.[6,7] It has been evaluated for its cognitive-enhancing properties in large clinical trials of AD, but the evidence of its efficacy is still controversial.[8,9] Vinpocetine, an alkaloid compound extracted from the periwinkle plant (*Vinca minor*), functions as a phosphodiesterase inhibitor. It is an effective vasodilator[10] and has been used in the prevention of cerebrovascular diseases[11] and in cognitive enhancement[12] and has also been shown to have anti-inflammatory and neuroprotective properties.[13] PET studies demonstrate vinpocetine's global uptake across the brain,[14,15] and its ability to effectively regulate blood flow and metabolism.[16] Taken together, the literature provides growing evidence that supports the efficacy of these plant-based extracts in exerting significant effects on cognition and blood flow when used individually. When they are combined with other BDNs, their synergistic properties offer the potential to exert more robust effects on brain function.

The current research team has previously demonstrated that a combination of BDNs resulted in significant improvements in regional cerebral blood flow (rCBF), mood, and cognition in a group of active and retired professional football players who showed signs of chronic traumatic brain injury (TBI) as a result of repetitive, subconcussive impacts to the head.[17] Specifically, the team reported global increases in rCBF as measured by single photon emission computed tomography (SPECT) imaging as well as significant improvements across several cognitive domains, including general cognitive function, general cognitive proficiency, reasoning, attention, and memory, as determined by the MicroCog neuropsychological assessment.

That prior study was an open-label design using BDNs comprised of a comprehensive, multiple vitamin/mineral complex (Table 1), omega-3 fatty acids, and a brain-enhancement supplement containing: (1) *N*-acetylcysteine (NAC)[18] and α-lipoic acid[19] to improve antioxidant levels, (2) phosphatidylserine to help regulate cortisol levels,[20] (3) huperzine A[21] and acetyl-L-carnitine (ALC)[22] to enhance acetylcholine availability, and (4) vinpocetine[15,23] and *Ginkgo biloba*[24] to enhance blood flow. In addition, the study included education on diet, exercise, weight loss, substance use, and sleep. The major limitation of this study's design was the fact that it did not use a randomized sample and was not placebo-controlled, and the results for the supplements were confounded by the simultaneous lifestyle interventions that alone could have accounted for the improvement.

In an effort to establish the efficacy of BDNs within a typical population, the current research team has investigated these nutrients in a double-blind RCT, at lower dosages than in its previous study and in the absence of lifestyle education or additional interventions. Lower dosages of the BDNs were used under the assumption that healthy individuals would exhibit fewer cognitive deficits and emotional impairments than professional football players and would require lower dosages to obtain a positive effect. The team measured cerebral perfusion (SPECT imaging), cognitive performance (MicroCog), emotional state (WebNeuro), and psychological well-being (BDI-II, BSI, QOLI) in BDNs- and placebo-treated groups at baseline, at 2 months following the first intervention, and at 4 months following a crossover intervention.

METHODS

Participants

This study was approved and monitored by the Lange Research Group Institutional Review Board. Fifteen adult men and 15 adult women were recruited from the community through local advertising to volunteer to participate in a randomized, double-blind, placebo-controlled, crossover study to demonstrate the clinical utility of BDNs. Each participant was educated on the study and gave written informed consent. Potential participants were screened for eligibility using (1) a detailed clinical history, which included both medical and psychiatric parameters; (2) the Structured Clinical Interview for DSM-IV (SCID) from Multi-Health Systems and American Psychiatric Press (North Tonawanda, NY, USA); (3) the Beck Depression Inventory-II (BDI-II) from Pearson (San Antonio, TX, USA); (4) the Brief Symptom

Table 1. NeuroVite Plus Composition (Four Capsules)

Ingredient	Compound	Dose
Vitamin A	Retinyl acetate	2500 IU
Vitamin A	β-Carotene	2500 IU
Vitamin C	Magnesium ascorbate	200 mg
Vitamin D_3	Cholecalciferol	2000 IU
Vitamin E	D-α-tocopheryl acid succinate, di-α-tocopheryl acetate	70 IU
Vitamin K_2	Menaquinone (MK-7)	45 mcg
Vitamin B_1	Thiamin mononitrate	15 mg
Vitamin B_2	Riboflavin	17 mg
Vitamin B_3	Niacinamide	50 mg
Vitamin B_5	D-calcium pantothenate	50 mg
Vitamin B_6	Pyridoxine HCL, pyridoxal-5-phosphate	20 mg
Vitamin B_{12}	Cyanocobalamin, methylcobalamin	500 mcg
Folic acid	5-methyltetrahydrofolic acid	400 mcg
Biotin		300 mcg
Choline	Bitartrate	55 mg
Calcium	Calcium carbonate	50 mg
Magnesium	Magnesium oxide, magnesium ascorbate	50 mg
Manganese	Manganese glycinate	5 mg
Zinc	Zinc glycinate	7.5 mg
Copper	Copper glycinate	1 mg
Chromium	Picolinate, histidinate, Chromax	200 mcg
Molybdenum	Molybdenum glycinate	50 mcg
Iodine	Potassium iodide	75 mcg
Selenium	SelenoExcell high selenium yeast	200 mcg
Lycopene	Tomato extract	3 mg
Lutein		3 mg
Quercetin	Quercetin dihydrate	30 mg
Broccoli sprout powder	Minimum 10% glucoraphanin	50 mg
Hesperidin complex		20 mg
Resveratrol		10 mg
Pterostilbene powder		20 mcg
Vanadium	Nicotinate glycinate chelate	25 mcg
Proprietary organic fruit and vegetable blend[a]		140 mg
Brain boosting blend[b]		575 mg
Full-spectrum digestive enzyme blend[c]		30 mg

[a]Wild blueberries, broccoli, tomato, spinach, acai, carrot, strawberry, alfalfa sprouts.
[b]Acetyl-L-carnitine, α-lipoic acid, phosphatidylserine, CoQ10.
[c]With lipase, amylase, lactase, cellulase, protease. Other ingredients: gelatin (capsule), dicalcium phosphate, microcrystalline cellulose, silica, magnesium stearate, calcium sulfate, β-cyclodextrin, starch, rice protein, palm oil, corn starch, sunflower oil, ascorbyl palmitate, lecithin, sodium copper chlorophyllin, titanium dioxide.

Inventory (BSI) from Pearson; and (5) the Quality of Life Inventory (QOLI) from Pearson.

The study excluded individuals who (1) had a current DSM-IV psychiatric disorder as assessed by clinical examination or SCID; (2) had a score greater than 21 on the BDI-II; and (3) were on psychiatric medications. Participants in the study were screened to exclude medical, neurological, and psychiatric conditions, including substance abuse. Of the 30 individuals recruited for the study who underwent the baseline evaluation, only 25 individuals completed the study. Five participants dropped out at various time points due to reasons unrelated to the study.

Those participants who passed the exclusion screening but who were taking nonpsychiatric medications, such as blood pressure or birth control pills, were asked to report any changes in dosage during the length of the study to the study's coordinator.

Design

After participants qualified to participate, they completed the following baseline measures: (1) technetium-hexamethylpropyleneamine oxime (Tc-HMPAO) brain SPECT imagaing, a measure of rCBF, occurs immediately after performing the Connors' Continuous Performance Task (CCPT-II) from Multi-Health Systems (North Tonawanda, NY, USA), a measure of attention and response inhibition; (2) MicroCog from Pearson, a computerized neuropsychological test that measures across nine cognitive domains; and (3) WebNeuro from Brain Resources (San Francisco, CA, USA), a computerized neuropsychological test that measures across 14 different domains in the areas of self-regulation, feeling, thinking, and emotion.

Following baseline testing, participants were randomized to the BDNs or placebo group by an independent administrator, ensuring that the research staff remained blind to each participant's intervention. Upon completion of the first intervention (BDNs or placebo) at the 2-month point, participants were retested using the baseline assessments, which included brain SPECT imaging, neuropsychological assessments (MicroCog and WebNeuro), and psychological inventories (BDI-II, BSI, and QOLI).

The participants were then assigned to the crossover intervention (BDNs or placebo treatment) for the final 2 months. Upon completion of the intervention, participants completed all of the above listed measurements in a final testing.

Intervention and Rationale

In both the first intervention and the crossover intervention, participants were administered BDNs or a placebo. The BDNs comprised three products.

Fish Oil. The dosage was 2.8 g of fish oil per day in two capsules, containing 860 mg of EPA and 580 mg of DHA. Omega-3 fatty acids have been demonstrated to improve memory, mood, and cognition, providing the rationale for inclusion of that supplement in the nutrient system.[25,26]

High-potency, Multiple Vitamin/Mineral Supplement. The dosage was four capsules. See Table 1 for the ingredients. This supplement was included because research supports the use of multivitamins to enhance mental performance.[27]

Brain-enhancement Supplement. Provided in four capsules, this supplement contained nutrients (1) to increase blood flow—80 mg *Ginkgo biloba*[24] and 10 mg vinpocetine[15,23]; (2) to modulate cortisol production—67 mg phosphatidylserine[20]; (3) to elevate acetylcholine levels—667 mg acetyl-L-carnitine[22] and 100 mcg huperzine A[21]; and (4) to enhance antioxidant production—200 mg α-lipoic acid[19] and 400 mg *N*-acetyl-cysteine.[18] Acetyl-L-carnitine, α-lipoic acid, *Ginkgo biloba*, *N*-acetyl-L-cysteine, and phosphatidylserine have been tested extensively and used in the supplement market; thus they are considered safe and effective for long-term use.

However, some of the ingredients in the study's brain-enhancement supplement that are newer to the market for nutritional supplements require some justification regarding dosage and safety. One of these ingredients was huperzine A, which was included in the brain-enhancement supplement at 100 mcg as the primary cognitive enhancer. Several meta-analysis studies[4,28] have been performed that demonstrate that dosages within the range of 200 to 400 mcg are considered safe and that they effectively improved general cognitive function at 6 weeks as assessed by the Mini-Mental State Examination (MMSE)[29] (Psychological Assessment Resources, Lutz, FL, USA).[30]

Another ingredient in that same supplement is vinpocetine, which was included at 10 mg to enhance cognition and improve blood flow to the brain. In a large, multicenter study, vinpocetine was administered (3 × 10 mg) to 203 patients with dementia and cognitive impairment, and a 16-week treatment protocol resulted in a significant improvement as measured by a cognitive performance scale.[12] In an RCT where 12 healthy female volunteers were administered vinpocetine or placebo in 10-, 20-, or 40-mg doses for 2 days, the researchers demonstrated that a 40-mg dose effectively improved memory as measured by the Sternberg Memory Scanning Task (SMS-Task),[31,32] a high-speed scanning in human memory, demonstrating the potential of this nutrient to work quickly in a short time frame if given at a sufficient dosage.[33]

And finally, a double-blind RCT studied the effects of *Ginkgo biloba*, another ingredient of the current study's brain-enhancement supplement, on 20 outpatients with Alzheimer's-type degenerative dementia. The study used a 240-mg dose over 3 months and resulted in improvements on the Syndrom-Kurz Test (SKT)[34] (Geromed GmbH, Spardorf, Bavaria, Germany), a neurocognitive test used to assess deficits of attention and memory and on functional EEG findings.[35] Given this evidence, the current research team concluded that these nutrients had the potential to exert measurable cognitive-enhancement effects and would be safe to be taken over 8 weeks. The team decided to use lower dosages of the individual ingredients, with the hypoth-

esis that they would act in a synergistic fashion with the other BDNs.

The placebo group received a combination of three supplements. The multiple vitamin/mineral complex and the brain enhancement supplement were comprised of rice flour, while the fish-oil control mixture of oils comprised olive oil (319 mg), soybean oil (262 mg), palm oil (205 mg), coconut oil (205 mg), canola oil (103 mg), natural lemon flavor (22 mg), and natural mixed tocopherols (3 mg).

ANALYSIS

Brain SPECT Imaging and ROI Analysis

Participants underwent high-resolution brain SPECT imaging to measure rCBF during a concentration task. Each participant received an age/weight-appropriate dose of technetium-99m hexamethylpropyleneamine oxime (Tc-99m HMPAO) intravenously in a dimly lit room while performing the CCPT-II. The radiopharmaceutical was injected 3 minutes after starting the 15-minute test. Participants were then scanned 30 to 45 minutes after injection using a Picker Prism 3000XP, triple-headed, gamma camera with low-energy, high-resolution, fan-beam collimators (Eclipse Systems, Inc, Bedford Heights, OH, USA). Data was acquired in 128 × 128 matrices, which yielded 120 images per scan, with each image separated by 3° and with a span of 360°. SPECT data was processed, smoothed, and corrected for attenuation using general linear (Chang) methods.[36] All images were reconstructed using an oblique reformatting program and oriented according to anterior-posterior commissure line, and the final images were similarly aligned for analysis.

Differences in Tc-HMPAO uptake in the whole brain and in regions of interest (ROIs) were analyzed using the Automated Anatomical Labeling (AAL) atlas,[37] which consists of 128 brain regions defined across both hemispheres, using ROI Extract software (Amen Clinics, Inc, Newport Beach, CA, USA). To minimize disruption to the individual scans caused by transforming the processed imaging data into stereotaxic space for ROI analysis, the ROI Extract software transforms the stereotaxic atlas into the individual scan space. The processed reconstructed images were left unchanged. Spatial normalization and alignment of the atlas to the individual space was performed using a mutual, information-based, affine registration[38] algorithm, implemented using the ITK Insight Toolkit (Kitware, Clifton Park, NY, USA). The mutual information-registration algorithm used in ROI Extract finds an affine transformation of a canonical SPECT scan template in Montreal Neurological Institute (MNI) space that minimizes the joint entropy between the unchanged individual image and the transformed atlas image. The resulting affine transformation matrix is then applied to the atlas label map, transforming it into the participant's specific space. After the atlas has been transformed into the individual space, ROI metrics can be calculated, including the region's mean, standard deviation, and minimum and maximum.

ROI means were analyzed using SPSS statistical software (SPSS Inc, Chicago, IL, USA) using a within-participant, repeated measures ANOVA that was modeled from baseline to treatment and back to placebo regardless of the actual randomized presentation order. The research team tested the hypothesis that treatment with BDNs would elicit a measurable response with a return toward baseline values upon administration of the placebo, independent of randomized order (significant quadratic response). In addition, the effect of treatment order was tested for each region as a between factor to ascertain whether treatment with BDNs had any carryover effect that might obfuscate treatment response. Post hoc, paired *t* tests were used for between-condition comparisons.

Inventory and Neuropsychological Tests Analyses

All measures were analyzed using a within-participant, repeated measures ANOVA that was modeled from baseline to treatment and back to placebo regardless of actual randomized presentation order. The research team tested the hypothesis that treatment with the BDNs would elicit a measurable response, with a return toward baseline values upon administration of the placebo, independent of randomized order (significant quadratic response). In addition, the effect of treatment order was tested for each measure as a between factor to ascertain whether treatment had any carryover effect that might obfuscate the treatment response. Post hoc, paired *t* tests were used for between-condition comparisons.

RESULTS

No participant dropped out due to side effects. Only one adverse incident was reported, which was dizziness. After the participant's blood pressure medication was adjusted downward, the dizziness was alleviated.

SPECT ROI Findings for rCBF

When compared to baseline, 30 brain regions showed significantly increased rCBF following the BDNs treatment versus placebo (Table 2). These areas included the prefrontal cortex, anterior and posterior cingulate, hippocampus, parahippocampus as well as regions in the occipital lobes (cuneus and calcarine), thalamus, and cerebellar region 10, Crus II, and vermis 9. Four other areas became significant when the effect of order was considered, including the right-frontal, midorbital region; left olfactory region; left insula; and right precuneus. This finding indicates that the effect of taking the BDNs in the study's first intervention period versus the second intervention period had a significant influence on the results.

Inventory and Neuropsychological Tests Results

The research team observed improvements for participants in the BDNs treatment across various domains of the psychological and neuropsychiatric assessments that were administered. See Table 3 for scores on assessments at baseline for the entire group and at the end of the study after the

Table 2. Significant ROI in the BDNs Compared to Placebo[a]

Region	Baseline	Placebo	BDNs	*P* Value
Frontal				
Frontal midorbital L	592.6	612.9	624.0	.014
Frontal midorbital R	588.9	603.4	619.1	.048[b]
Frontal inferior gyrus R	492.3	504.9	509.1	.037
Frontal mid L	479.0	493.7	510.9	.048
Olfactory L	554.4	588.5	593.4	.044[b]
Insula L	620.3	653.2	662.0	.045[b]
Cingulate				
Cingulate, anterior L	612.5	628.7	649.2	.012
Cingulate, anterior R	559.5	590.2	601.8	.021
Cingulate, posterior L	612.5	628.7	649.2	.027
Cingulate, posterior R	559.5	590.2	601.8	.044
Temporal Lobe				
Hippocampus L	534.1	564.8	577.2	.019
Hippocampus R	549.7	577.1	585.6	.047
Parahippocampus L	471.0	498.3	503.7	.046
Temporal mid L	533.8	556.4	565.6	.039
Temporal inf post R	401.7	404.4	420.2	.036
Temporal mid post L	536.6	554.1	564.8	.037
Temporal mid post R	476.4	487.1	501.2	.041
Occipital-Parietal Lobe				
Precuneus R	539.9	572.0	574.0	.038[b]
Cuneus L	528.0	551.0	558.9	.029
Cuneus R	520.9	542.9	551.8	.012
Calcarine L	542.1	555.3	568.9	.031
Calcarine R	576.7	599.9	613.6	.038
Occipital inf R	332.7	331.7	338.9	.046
Occipital mid L	457.7	464.2	472.5	.030
Occipital mid R	389.1	396.1	406.1	.030
Occipital sup R	422.6	441.5	448.8	.047
Cerebellum				
Cerebellum 10L	382.7	381.2	405.4	.018
Cerebellum Crus II	364.2	353.5	375.2	.032
Vermis 9	706.2	747.0	775.0	.049
Thalamus				
Thalamus L	653.7	686.0	701.2	.009

[a]The mean rCBF for each region of interest (ROI) is given based on condition (baseline, placebo, BDNs), reaching significance at $P = .05$ (n = 25). *P* values are independent of randomized order and represent a significant quadratic response.
[b]Demonstrates significance upon accounting for the effect of order, indicating that the order of treatment had an influence on the results.

Table 3. Significant Neuropsychological Outcomes for BDNs Compared to Placebo[a]

		At End of Study After Crossover Intervention		
Measure/Scale	**Baseline**	**Placebo**	**BDNs**	***P* Value**
Executive function (WN)	5.06	7.13	7.50	.002[b]
Reasoning (MC)	31.95	47.71	50.29	.008[b]
Memory (MC)	53.19	76.79	78.63	.014[b]
Information processing efficiency (WN)	5.88	6.22	6.81	.015[b]
Depressed mood (WN)	6.03	6.00	6.79	.017[b]
Emotional identification (WN)	4.74	4.29	5.15	.041[b]
Positive symptom total (BSI)	15.65	13.71	11.81	.024[b]
Information processing accuracy (MC)	43.50	55.75	58.54	.027[b]
Hostility (BSI)	0.296	0.313	0.174	.052/.018[c]

Abbreviations: MC = MicroCog; WN = WebNeuro; BSI = Brief Symptom Inventory.

[a]The mean score for each neuropsychological assessment measure is given based on condition (baseline, placebo, BDNs), reaching significance at $P = .05$ (n = 25). *P* values are independent of randomized order and represent a significant quadratic response.
[b]Demonstrates significant improvements for BDNs as compared to placebo.
[c]Demonstrates significance upon accounting for the effect of order.

crossover intervention for treatment with the placebo versus BDNs.

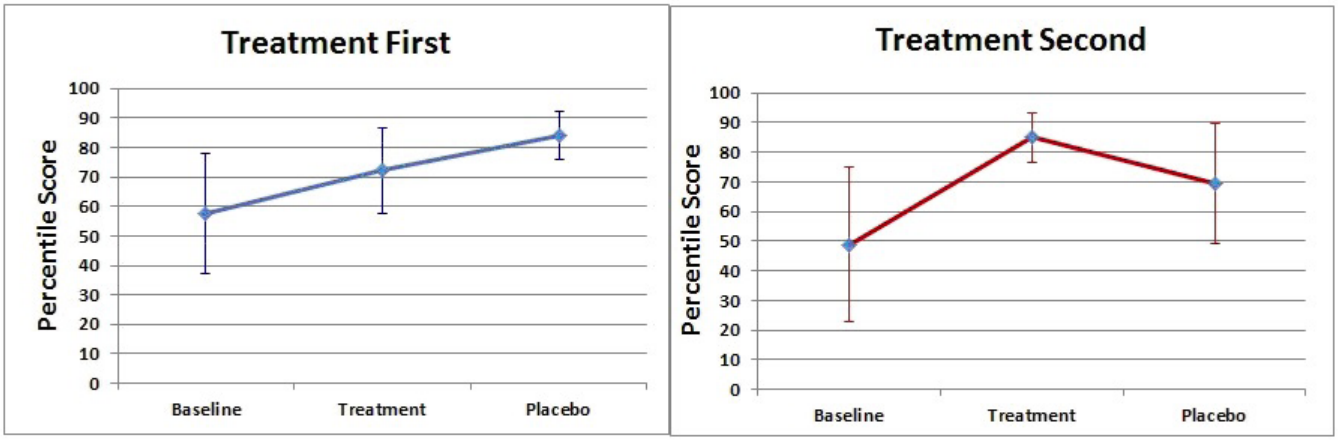
Significant improvements were observed for the BDNs: (1) on the MicroCog—reasoning, $P = .008$, memory, $P = .014$, information processing accuracy, $P = .027$; (2) on the WebNeuro—executive function, $P = .002$, information processing efficiency, $P = .015$, depressed mood, $P = .017$, and emotional identification, $P = .041$; and (3) on the BSI—positive symptom total (PST), $P = .024$ and reduced hostility, $P = .018$. For the last, significance occurred upon accounting for the effect of order.

BSI. On the BSI, treatment with BDNs demonstrated a significant improvement on the positive symptom total (PST), which is an index of the number of symptoms a participant is experiencing across nine different scales (somatization, obsessive-compulsive, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, and psychoticism). The higher the score is, the greater is an individual's experience of the symptoms of distress. The baseline mean score for all participants was 15.65 ± 1.96 SEM, while treatment with placebo revealed a slight reduction in symptoms at 13.71 ± 1.86 SEM and treatment with BDNs demonstrated an even greater reduction at 11.81 ± 2.15 SEM ($F_{1,22} = 5.29$, $P = .024$).

Interestingly, the scores on the Hostility Scale (a subscale of the PST) improved and trended toward significance, with the baseline mean for all participants at 0.296 ± 0.052 SEM, while treatment with placebo showed an elevated score at 0.313 ± 0.095 SEM and treatment with BDNs revealed a decrease in the score to 0.174 ± 0.046 SEM ($F_{1,22} = 4.22$, $P = .052$). While the score on the hostility scale did not initially achieve significance with BDNs treatment, it did reach significance when the effect of order was considered ($P = .018$). This finding indicates that the order in which the participants received the interventions had a significant impact on the results. The scores across the other scales improved during BDNs treatment as compared to the placebo, but the differences did not reach significance, which may have resulted from the fact that this cohort included healthy participants.

WebNeuro. The research team observed several improved scores on the WebNeuro assessment, which measures across four primary domains—self-regulation, feeling, thinking, and emotion—on a scale of 0 to 10 (low functioning/clinically significant to high functioning/superior ability). A significant increase in executive function (an index within the thinking domain), or the ability to plan, monitor, adjust, and organize behavior to meet goals, was observed with BDNs. The mean at baseline for all participants was 5.06 ± 0.591 SEM; at the end of the study was 7.13 ± 0.654 SEM for treatment with placebo and 7.50 ± 0.524 SEM ($F_{1,15} = 27.2$, $P = .002$) for treatment with BDNs. In addition, BDNs improved

Figure 1. Memory Response to Treatment with BDNs[a]



[a]Interaction of treatment order and treatment condition for memory; means and 95% confidence intervals are shown. The response is linear when BDNs are given first compared to a quadratic response when BDNs are given second, regressing from baseline to treatment to placebo.

information processing efficiency (also within the thinking domain), or the ability to process complex information under time demands, which requires a balance of focus and flexibility. The mean at baseline for all participants was 5.88 ± 0.311 SEM; at the end of the study was 6.22 ± 0.442 SEM for treatment with placebo and 6.81 ± 0.313 SEM for treatment with BDNs ($F_{1, 15} = 7.63$, $P = .015$).

A significant improvement was observed on the depressed mood scale (an index within the feeling domain), which ranges from feeling extremely low (score of 0) to an absence of sadness (score of 10). The mean at baseline for all participants was 6.03 ± 0.336 SEM; at the end of the study, treatment with placebo maintained the mean at 6.00 ± 0.466 SEM, while treatment with BDNs improved the mean to 6.79 ± 0.376 SEM ($F_{1, 16} = 7.1$, $P = .017$). These results indicate that while taking BDNs, participants were less likely to experience feelings of sadness.

And finally, treatment with BDNs demonstrated an improved score on the emotional identification scale (an index within the emotion domain), which measures the ability of a participant to identify basic facial expressions of emotion, such as fear and happiness. The mean at baseline for all participants was 4.74 ± 0.470 SEM; treatment with placebo decreased the mean to 4.29 ± 0.597 SEM and treatment with BDNs significantly increased the mean to 5.15 ± 0.627 SEM ($F_{1, 16} = 4.91$, $P = .041$). These results suggest that participants on BDNs experienced a higher level of emotional functioning. See Table 3 for the results.

MicroCog. Significant improvements were observed on the MicroCog, a neurocognitive assessment measuring across nine domains (general cognitive functioning, general cognitive proficiency, information processing accuracy and proficiency, attention, reasoning, memory, spatial processing and reaction time) on a percentile scale of 0 (low functioning) to 100 (high functioning). Treatment with BDNs resulted in improved scores in reasoning, memory, and information processing accuracy and, furthermore, demonstrated an effect of order with respect to general cognitive proficiency. The mean at baseline for all participants on reasoning (consisting of three subtests, including verbal analogies, object match, and math) was 31.95 ± 5.53 SEM; at the end of the study treatment with placebo was 47.71 ± 5.88 SEM and treatment with BDNs was 50.29 ± 6.13 SEM ($F_{1,20} = 8.55$, $P = .008$). The mean at baseline score for memory was 53.19 ± 7.19 SEM; at the end of the study treatment with placebo was 76.79 ± 4.12 SEM and treatment with BDNs was 78.63 ± 4.12 SEM ($F_{1, 20} = 7.24$, $P = .014$). The mean at baseline score for information processing accuracy was 43.50 ± 5.95 SEM; at the end of the study treatment with placebo was 55.75 ± 5.40 SEM and BDNs was 58.54 ± 4.60 SEM ($F_{1, 20} = 6.02$, $P = .027$). See Table 3 for a list of the results.

Treatment with BDNs interacted significantly with order of administration for general cognitive proficiency ($F_{2,38} = 3.995$, $P < .027$), which was consistent with significant interactions with order and condition in its composite measures, memory ($F_{2,38} = 5.768$, $P < .006$) and information processing speed ($F_{2,38} = 4.205$, $P < .022$). The graph of memory illustrates the interaction, revealing a linear relationship when treatment was given immediately after baseline, with improvements continuing into the placebo period. When treatment was given last, the relationship was quadratic, indicating that treatment led to a significant increase over both baseline and placebo, even after repeated testing across 4 months (Figure 1).

DISCUSSION

Compared to placebo, treatment with BDNs increased rCBF across 30 ROIs, particularly in the prefrontal cortex, anterior and posterior cingulate gyrus, hippocampus, and cerebellar region 10, Crus II, and vermis 9. Crus II is a phylogenetically younger part of the cerebellum and has been associated with higher cognitive functions, such as working memory and executive control,[39] as is the prefrontal cortex,

anterior cingulate, and hippocampus.[40,41] The posterior cingulate is involved in integrating visual memory and remembering familiar people and is one of the first areas reported to have decreased perfusion, together with the hippocampus, in Alzheimer's disease.[42,43]

The improvements observed in the PST within the BSI assessment suggest an improvement in psychological well-being with BDNs. Within this assessment, the hostility scores became significant when considering the effect of order of treatment. This finding suggests that the order in which the supplements were taken was important, and it may indicate a carryover effect when individuals took the BDNs first.

Results from the WebNeuro assessment included improved executive function and information processing efficiency together with an improvement in mood and in emotional identification. The MicroCog results showed significant improvement in reasoning, memory, and information processing accuracy. Furthermore, interactions between treatment order and treatment condition in the cognitive measures indicated that treatment with BDNs may have a lasting effect on the brain, even after withdrawal of treatment. However, the possibility also exists that some of the components of the BDNs may remain in the system after withdrawal. To further distinguish carryover effects due to lasting changes in brain function from the effects of accumulation of BDNs in the body, future studies of this formulation will include a sufficient washout period between treatment phases. Adding a second treatment phase as a repeated crossover intervention after a washout period would also more definitively show the relationship between BDNs and cognition. More specifically, showing that withdrawing active treatment causes a return toward baseline performance and that reintroducing the treatment after withdrawal once again causes a increase in positive effects would demonstrate that carryover of positive affects is limited and maintaining improvements are tied to active treatment.

Taken together, this study demonstrates that the unique mixture of compounds in the BDNs had a synergistic effect and resulted in improvements on rCBF and across multiple measures of neuropsychological function, specifically in the areas of executive function, reasoning, information processing efficiency and accuracy, and emotional identification as compared to placebo. The improvements observed on both the PST and the depressed mood scale indicates the possibility of an enhanced sense of psychological well-being.

This study is timely as the market for brain health supplements is growing at a rapid pace, despite skepticism about the overall benefit of single or multiple-ingredient products[44] and conflicting studies, with some showing significant benefits[27,45] and others showing limited or no benefits for ingredients commonly perceived to be helpful.[46-48]

Perhaps one reason why many nutrients do not show consistent effectiveness is that they are used in isolation to target only one pathological system in the brain,[49] such as low blood flow, inflammation, or low levels of neurotransmitters, such as acetylcholine. This study used nutrients targeted at multiple mechanisms, including vitamin-and-mineral nutrient status (multiple vitamin/mineral supplement), omega-3 fatty acid levels (fish oil), blood flow (*Ginkgo biloba*, vinpocetine), acetylcholine levels (acetyl-L-carnitine and huperzine A), cortisol levels (phosphatidylserine), and antioxidant status (*N*-acetyl-cysteine and α-lipoic acid). In addition, omega-3 fatty acids[50] and vinpocetine[51] have been shown to have anti-inflammatory effects.

The primary limitation of this study was that the cohort was comprised of healthy individuals, so that most neuropsychological measures, even those that demonstrate significant improvement, started in the normal range. A second limitation was that the research team did not provide for a washout period between treatments. The team's initial rationale was that pharmacokinetic data on the half-life and clearance rate of each ingredient demonstrated no indication of a carryover effect within the study's participants between the two phases of the study. However, the data indicated that a carryover effect occurred across some variables (brain regions and neuropsychological measures) which requires further investigation. One of the concerns with nutritional interventions is that a rapid return to baseline occurs; therefore, the advantage to the current study's design was that it allowed for the measurement of these variables.

CONCLUSION

In conclusion, the research team demonstrated that use of BDNs had the ability to improve blood flow to the brain and may function to enhance psychological well-being in healthy individuals. The team acknowledges that this study does not give adequate information on the effect of these BDNs in a clinical population.

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Daniel G. Amen, MD, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

AUTHOR DISCLOSURE STATEMENT

Daniel G. Amen, MD, is the sole owner of Amen Clinics, Inc, which designed and produces the product studied. Kristen Willeumier, PhD; Derek Taylor; Kristine Ojala; and Jasleen Kaur are employees of Amen Clinics, Inc.

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