A Randomized, Placebo-Controlled Clinical Trial of a Novel Dietary Supplement (Braini) on Standardized CNS Vital Signs Cognitive Performance Parameters in Adults

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Abstract

Objective: To test the effectiveness of a novel dietary supplement as a support for cognitive function in healthy younger and older adults

Design: A double-blind, randomized, placebo-controlled trial of the dietary supplement, Braini® in two age cohorts with 60 participants: 31 healthy younger adults (18–30 years) and 29 healthy older adults (55–80 years).

Intervention: A 28-day intervention of a dietary supplement (active or placebo) taken daily with cognitive assessment using CNS Vital Signs computer-based testing at day 0 and 28. Participants were asked to fill out a daily survey regarding compliance with supplement protocol, changes in health, adherence to the protocol, and reported side effects. CNS Vital Signs provides aged normed aggregated outcome measures for Processing Speed, Psychomotor Speed, Reaction Time, Cognitive Flexibility, Executive Function, and Motor Speed.

Results: Significant improvements in performance were found for two CNS Vital Signs domains, Cognitive Flexibility (p = 0.048), and Executive Function (p = 0.025) in the treated younger adults (n = 12) compared with the placebo group (n = 19) at day 28 compared with baseline. The Shifting Attention Test Reaction Time (SAT-RT), a measure of shifting attention correct response reaction time, showed significant improvement at 28 days in those taking Braini in both younger (p = 0.004) and older adult cohorts (p = 0.05) with an average improvement over the control subjects of 44%. No serious side effects were reported.

Conclusions: The dietary formulation, Braini, safely and significantly improved cognitive flexibility and executive function in younger adults and trended positively in older adults in this study that was stopped prematurely due to pandemic restrictions. Scores on SAT-RT significantly improved in both younger and older adults. Further studies are needed to confirm that Braini reliably improves cognitive function in additional CNS domains in healthy adults (Clinicaltrials.gov under registration number: NCT04025255).

Keywords: cognitive flexibility, executive function, dietary supplement, Peptylin, Braini
Introduction

The number of people affected by cognitive decline continues to rise as the U.S. population ages and this decline can be seen as early as age 45. Optimizing cognitive function performance in the areas of psychomotor speed, reaction time, cognitive flexibility, executive function, processing speed, and motor speed during adulthood is a key mechanism to combat cognitive decline as one reaches older adulthood.2–6

Human clinical studies in Korea have demonstrated that orally ingested active silk fibroin peptide, exclusively licensed in North America as Peptylin, improves memory and cognitive performance scores up to 30%–40% in young and older cohorts in 30-day trials.5–17 Although the exact mechanism of action for these clinical improvements has not been elucidated, active acetylcholinesterase inhibition, Peptylin's potent antioxidant activity, enhanced synaptogenesis, and down-regulated amyloid beta aggregation are likely mechanisms.10,13,17 Significantly, the synergistic effect of the patented Braini formulation demonstrated superior benefits.18

Peptylin was developed under the leadership of Dr. Sung-Su Kim and is one of the important ingredients of the Braini formulation. In 2018, Famenity received a US FDA New Dietary Ingredient Notification (NDIN) for Peptylin. Sustained memory enhancement from daily intake, with overall safety and tolerability, are the hallmarks of ingested Peptylin in Korea. In 2020, Braini received a US FDA NDIN 1137.

Braini is a proprietary nutraceutical formulation designed to support neural cell viability, signaling efficiency, and protection from pro-inflammatory and oxidative stressors.7–17 Braini contains refined Buglossoides arvensis seed oil. This dietary oil is unique among the plant-based omega fatty acid rich plant-derived oils for having the highest combined omega-3 alpha linolenic acid (ALA, c18:3, n-3), stearidonic acid (SDA, c18:4, n-3), and gamma-linolenic acid (GLA, c18:3, n-6) content. Licensed exclusively to Braini LLC as Neurxcel, this oil has EU Novel Food status as of 2015 and Canadian Novel Foods status as of 2020.

The other active ingredient in the Braini formulation is North American wild blueberry (Vaccinium corymbosum) powder (minimum 12% anthocyanins). Wild blueberry is a common food ingredient and has been shown to support cognitive function in previous studies.19–22

Several clinical trials have studied these separate nutraceutical ingredients for improved memory enhancement and cognitive performance. Of those conducted, all formulations were well tolerated by participants.18,23–27 Clinical trials using these key ingredients individually (Peptylin, Neurxcel and wild blueberry) have measured the effects of these active ingredients and have been shown to support anti-inflammatory, neuro-protective and cognitive performance benefits.28–30

The Braini formulation is novel and harnesses synergistic effects. In vitro cell challenge assays of the active constituents in Braini were performed to assess cell viability and anti-oxidant activity. Braini showed significant improvement in cell viability and inhibition of reactive oxygen species. These unpublished results (from Dr. E. Hernandez at Innopro S.L.) highlight a possible mechanism for the support of cognitive function tested in this study and are the subject of patent and a pending patent application.18,31

Based on existing science supporting the neuroprotective role of Peptylin, the Braini formulation may be an effective dietary supplement for improving cognitive function and preventing cognitive decline stemming from oxidative challenges. Oxidative challenges are commonly associated with neurotransmitter viability and performance, which can lead to dementia and Alzheimer's disease.28 The Braini formulation shows promise as a natural therapeutic candidate to slow or prevent damaging neurophysiologic effects.

The purpose of this randomized double-blinded, placebo-controlled study with two age cohorts was to determine whether the novel dietary supplement formulation of Braini, when orally ingested as directed, demonstrates improvements in standardized cognitive performance tests compared with a control population in both younger and older adults with normal cognitive function.

Materials and Methods

Participants

Cohort A participants (n = 38) were younger adults (18–30 years), and Cohort B participants (n = 36) were older adults (55–80 years). The trial was conducted on a university campus in a quiet office area. It was stopped early due to the coronavirus pandemic when the university campus was closed to visitors. A sample size of 50 in each cohort was determined by a power calculation. The planned subject enrollment was modified due to campus closures.

Study eligibility required participants to: (1) continue with their current diet and refrain from taking any new nutritional or herbal supplements; (2) have English language proficiency; (3) not be clinically diagnosed with Alzheimer’s disease, stroke, Parkinson’s, or dementia; (4) not have active fibromyalgia, multiple sclerosis, seizures/epilepsy, or other known diseases that may affect memory or cognition; (5) not have taken prescription drugs to support memory/prevent cognitive decline in the past 180 days nor to treat other diseases referenced earlier in criterion number 4; (6) not have taken dietary supplements that include wild blueberry extract, Neurxcel, or silk protein peptide in the past 90 days; (7) not have taken any medicines that are stimulants; (8) not have GI disorders known to impair absorption of nutrients; (9) not have traumatic brain injury in their personal history; (10) not be participating in another clinical trial; (11) not be pregnant; and (12) have a current Montreal Cognitive Assessment (MoCA) score of >21.

Standard protocol, recruitment, and informed consent

Informed consent was obtained from all participants, and methods were carried out in accordance with protocols approved by the University of North Carolina Asheville (UNCA) Institutional Review Board. Participants were recruited from the UNCA, from the Osher Lifelong Learning Institute (OLLI) at UNC Asheville, and from the surrounding area from November 2019 through February 2020. Participants were offered $50 for study completion, and month supply of the Braini dietary supplement was offered at the conclusion of the study to participants who were allocated to the placebo control group.
**Study design**

A randomized, double-blinded, placebo-controlled dietary supplement study with younger and older adult cohorts comparing Braini dietary supplement and a placebo was conducted between November 2019 and April 2020. Interested individuals were screened for eligibility by phone. If eligible and still interested, participants reviewed and signed a detailed informed consent form, completed a demographics and health behaviors questionnaire, a health history form, and the MoCA Test intake questionnaire.

When cleared for participation, they did a practice set of computer-delivered online cognitive assessments using CNS Vital Signs (Morrisville, NC, USA). This neurocognitive performance testing procedure is internationally recognized as a standardized neurocognitive performance assessment tool (https://www.cnsvs.com/).

Participants were randomly assigned stratified by age cohort to either the Braini supplement or placebo group using block randomization with groups of 4 to achieve a 1:1 allocation by a research colleague. Sequentially numbered containers were assigned by A.C.M. who also enrolled the participants. Both experimenters and participants were blinded to group assignment.

A series of CNS Vital Signs cognitive assessments were given, which included psychomotor speed, reaction time, processing speed, cognitive flexibility, executive function, and motor speed. The participants were given a practice test and within 1 week they took a baseline test. Then after consuming the active or placebo supplement for 28 days, they took their final test. All testing was completed on the same computer in private spaces at the university.

**Nutritional supplement and placebo intervention**

Braini is a proprietary nutraceutical formulation comprising ingredients generally recognized as safe (GRAS) or registered by the FDA. The daily dose of Braini contains 400 mg of Peptylin; Neurxcel oil delivered as 500 mg of micro-encapsulated powder (containing 250 mg Neurxcel oil), in a cornstarch and corn syrup solids powder base carrier; and 100 mg wild blueberry powder. All active ingredients are at safe concentrations.

The placebo (rice starch) utilized in this clinical trial was formulated by a Good Manufacturing Practice-compliant dietary supplement contract manufacturer and made to be as similar as possible to the active dietary supplement in appearance, odor, and other key characteristics.

Participants were randomized into either active or placebo supplement groups and were instructed verbally and in writing to consume 2 capsules per day of the product they received for a total of 56 capsules over the 28-day intervention period.

Each day during the trial, study staff sent a text or an email to participants with a link to a form to remind and ask participants whether they took their supplements that day. The email communication also prompted participants to respond on whether they had experienced any changes in health or health behaviors. Compliance was measured from the daily surveys (98.4% of respondents reported taking their supplements daily). The participants were instructed to report any adverse events to the study staff immediately and were reminded to do so on each daily survey.

At each visit (day = 0 and day = 28), study staff asked participants whether they had experienced any adverse events, changed their dietary habits, or experienced any other notable changes to their lifestyles. If participants reported an adverse event, this was reported to the study physician (94.8% of survey responses reported no concerns on their daily forms). The concerns that were noted were metallic taste in mouth, trouble concentrating, light-headedness, vertigo, difficulty sleeping, headaches, and feeling flushed. No serious adverse events were expected or observed.

**Cognitive assessment**

Our primary outcome measure was a suite of computer-based tests established and validated by CNS Vital Signs. They provide a computer-based 30-minute assessment suite measuring neurocognitive performance parameters to provide aggregated outcome measures for Processing Speed, Psychomotor Speed, Reaction Time, Cognitive Flexibility, Executive Function, and Motor Speed. A comprehensive reliability and validity analysis of the CNS Vital Signs platform was published in 2006 in the *Archives of Clinical Psychology*. The MoCA was used for screening to exclude any participants with cognitive impairment.

**Statistical analysis**

CNS Vital Signs outcome measures for Processing Speed, Psychomotor Speed, Reaction Time, Cognitive Flexibility, Executive Function, and Motor Speed, along with the correct response Shifting Attention Test Reaction Time (SAT-RT), were assessed. The difference score was calculated by subtracting the post-intervention CNS Vital Signs domain scores from the baseline CNS Vital Signs domain scores. These standard scores have a mean value = 100 and standard deviation = 15.

We analyzed the SAT-RT as a raw score in milliseconds to evaluate changes on executive reaction time. The CNS Vital Signs difference scores were then used for analyses to reflect change from baseline. Independent-samples t-tests with bootstrapping (1000 samples at 95% confidence interval) were utilized. Levene’s test was not significant, so equal variances were assumed.

In addition, due to the small and unequal sample size for the groups, nonparametric Mann–Whitney U tests were also used and confirmed the findings of the independent-samples t-tests. The results were analyzed using the statistical package SPSS v.27. For the older adult cohort, the analysis of the active Braini and placebo for all CNS tests and the SAT-RT analysis was done by the pharmaceutical consulting firm Pharma Initiatives (Chapel Hill, NC, USA) using R Studio Version 1.1.456 software (RStudio, Inc.).

**Results**

**Participants**

Thirty-eight younger adults enrolled in the study. One withdrew, and four did not finish. Thirty-three younger participants completed the study. Thirty-six older adults enrolled in the study. Three withdrew, and two did not finish. Thirty-one older adults completed the study. In addition, four participants, two in the younger cohort and two
in the older cohort, had invalid CNS Vital Signs data. Analysis was performed on 31 young adults ($n=12$ treatment; $n=19$ placebo) and 29 older adults ($n=13$ treatment; $n=16$ placebo).

Reasons for participant withdrawal: vertigo ($n=1$), urinary tract infection ($n=1$), unspecified no show on day 28 ($n=1$), and difficulty concentrating ($n=1$). Only subjects who completed the study with valid data on both the pre- and post-test were analyzed. See Figures 1 and 2 for CONSORT flow diagrams. Participant demographics and health behaviors are presented in Table 1.

Young adult cohort
In healthy younger adults ages 18–30, participants taking Braini for 28 days experienced significant improvement

![FIG. 1. CONSORT diagram for participant recruitment to study completion for young adult cohort.](image)
versus placebo, significant differences were found for two CNS Vital Signs measurements, Cognitive Flexibility [mean difference 9.715, \( t(29) = 2.151, p = 0.040 \)], Executive Function [mean difference 7.745, \( t(29) = 2.190, p = 0.037 \)]. For both of these domains, a larger difference score represented improved performance at post-test compared with pre-test or baseline (Fig. 3a).

Significant differences were also found in the component measure SAT-RT (mean difference 83.285 \( t(29) = 3.131, p = 0.004 \)), with the treatment group \( n = 12 \) having improved performance compared with the placebo group \( n = 19 \) at post-test compared with baseline. For SAT-RT, the treatment group’s mean difference score was 87.917±78.885 and the placebo group’s mean difference score was 4.632±67.689. This is a raw score in milliseconds; thus, a larger number indicates a faster reaction time at post-test compared with baseline (Fig. 4).

Cohen’s d was calculated and resulted in \( d = 0.707 \) for the group difference on Cognitive Flexibility, \( d = 0.807 \) for the group difference on Executive Functioning, and \( d = 1.154 \)

**FIG. 2.** CONSORT diagram for Healthy Older Adults cohort.
**Table 1. Baseline Demographic and Behavior Information**

<table>
<thead>
<tr>
<th></th>
<th>Study A: Younger cohort</th>
<th>Study B: Older cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Braini supplement (n = 12)</td>
<td>Placebo (n = 19)</td>
</tr>
<tr>
<td>Age (mean years)</td>
<td>20.6</td>
<td>21.6</td>
</tr>
<tr>
<td>MOCA score (mean)</td>
<td>24.7</td>
<td>25.1</td>
</tr>
<tr>
<td>Gender</td>
<td>8 F</td>
<td>14 F</td>
</tr>
<tr>
<td></td>
<td>4 M</td>
<td>5 M</td>
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<tr>
<td>Exercise (% yes)</td>
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<td>74</td>
</tr>
<tr>
<td>Caffeine use (% yes)</td>
<td>42</td>
<td>74</td>
</tr>
<tr>
<td>Tobacco use (% yes)</td>
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<td>5</td>
</tr>
<tr>
<td>Alcohol use (% yes)</td>
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<td>89</td>
</tr>
<tr>
<td>Mental health concern (% yes)</td>
<td>42</td>
<td>63</td>
</tr>
<tr>
<td>Trouble sleeping (% yes)</td>
<td>—</td>
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</tr>
</tbody>
</table>

MOCA, Montreal Cognitive Assessment.

Measures of executive function translate to valuable everyday tasks such as managing time effectively, paying attention to and sequencing instructions correctly using short-term memory, switching focus between activities requiring memory, planning and organizing activities, recalling details, and thinking creatively. Braini showed significant improvements in Executive Function and Cognitive Flexibility domains.

CNS Vital Signs identifies Executive Function and Cognitive Flexibility as testing parameters for attention deficit disorder, attention deficit/hyperactivity disorder, non-amnestic mild cognitive impairment (MCI), sleep, depression, chemotherapy, brain, and concussion. In addition, Cognitive Flexibility is used as a testing parameter for MCI, early dementia.

The SAT-RT correct response measurement is a key component of the CNS Vital Signs Executive Function outcome score. SAT-RT improvement creates safer environments where one’s capacity to respond in shifting attention environments (e.g., driving an automobile, or playing sports) with more efficiency, speed, and accuracy can be vital.

Among younger adults, a significant difference in executive function from baseline to 28 days was observed between Braini and placebo. The fact that any statistically significant differences were found with small cohort sizes (<20 individuals per arm) is remarkable, especially with executive function in healthy young subjects whose brains are generally at their physiological peak in terms of brain mass and integrity over their entire normal life course. These differences were noted in the absence of any other controlled variables involving diet or lifestyle behaviors (i.e., in free-living participants).

In addition, clinically relevant increases in cognitive flexibility were observed in younger adults taking Braini compared with those receiving placebo. Measures of cognitive flexibility translate to skills such as reasoning, decision making, impulse control, and strategy formation. This supports a person’s ability to adapt to changes in complex sets of instructions.

Improvements in executive functions and cognitive flexibility allow people to focus attention on specific tasks, successfully solve problems, and plan ahead; thus, these are important for everyday life.
FIG. 3. (A, B) Differences between the baseline and the final scores after 28 days are presented for both the younger (A) and the older (B) cohorts for all six tests. These results demonstrate the significant improvement in executive function and cognitive flexibility for the younger adults consuming the Braini supplement. For both domains, a larger difference score represented improved performance at post-test compared with pre-test or baseline.
Limitations

Study strengths include participants who largely complied with the study protocol and experienced no serious side effects, suggesting that the Braini supplement was well tolerated. And the CNS Vital Signs testing protocol is validated and well studied. Study limitations include testing at 2 time points (day 0 and 28) rather than 3 or 4. This means that a single invalid test forced the participant to be dropped from the analysis.

A further limitation in the older adult cohort was the “ceiling effect” noted, which is likely due to the population enrolled. The OLLI @ UNCA attracts many retired professionals with advanced degrees, which may have led to the above average baseline scores. Another limitation was the study time period being cut short by the pandemic (UNCA closed to visitors while the study was underway). Based on our power analyses, we anticipated needing 50 participants in each age cohort with random assignment into placebo and control groups of ~25 participants each. Instead, we had to stop the study when we had enrolled 74 participants totally (38 and 36 in younger and older adult cohorts, respectively) and some of these enrolled participants were not able to complete their 28-day test (6)—leading to smaller than planned sample sizes.

All three ingredients have peer-reviewed evidence of positive effects on known factors affecting neurotransmitter function and/or physiological responses to oxidative and inflammatory stressors.10,28,35,36 The Braini supplement appears to act rapidly (4 weeks) to cross the blood-brain barrier and supply important “building blocks” for enhanced neurotransmitter support and/or assistive anti-oxidative and/or anti-inflammatory activities that result in measurable benefits in cognitive functioning.

Additional placebo-controlled trials involving larger cohort sizes and biomarkers confirming changes in neurophysiological capacity are warranted. Additional research may elucidate and identify possible mechanisms of action and guide researchers toward safe, effective, and natural alternative dietary interventions that do not incur the known side-effects nor the societal infrastructure costs of conventional pharmaceutical interventions.

Conclusion

Daily use of the novel dietary supplement, Braini, taken for 4 weeks significantly increased cognitive flexibility and executive function among younger generally healthy adults compared with a placebo. Executive Function Shifting Attention Test (SAT RT) correct response reaction time was clinically and statistically significant creating improvement in both younger and older adults.

This combination of safe dietary ingredients shows promise for supporting cognitive function among adults as measured by CNS Vital Signs and may indicate support for improved efficiency of mental processes and a healthier brain. Further studies that are representative of the general population and adequately powered are needed to confirm this finding and to better understand the effectiveness of this brain supplement in supporting cognitive function.

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Authors’ Contributions
A.J.L. led the study design, IRB negotiations; participated in data collection; and drafted and finalized the manuscript. A.C.M. participated in the study design, led the data collection, and reviewed the manuscript. B.D.H. conducted the analysis of the results, participated in drafting the manuscript and the presentation of the results, and consulted on the use of CNS Vital Signs. S.S.K. consulted on the use of the active ingredients in Braini but did not have a role in data collection or analysis. P.H. participated in study design, dietary supplements manuscript drafting, and led the review and editing of the manuscript.

Author Disclosure Statement
S.S.K. is an owner in Braini, LLC. The other authors have no conflicts of interest to report.

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