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CLINICAL TRIAL STUDY

An Open-label Exploratory Study Investigating BDNF Essentials® on Cognition in Healthy Adults with Self-reported Memory Complaints

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Abstract: ***Background*:** An open-label, exploratory study has been conducted to investigate the efficacy of BDNF Essentials® for cognitive function and neurological health.

***Methods*:** Twenty healthy adults ≥45 years of age, with a score ≥24 on the Mini-Mental State Examination (MMSE-2) and self-reported memory complaints were supplemented with BDNF Essentials® for 84 days. Computerized Mental Performance Assessment System (COMPASS) tests for memory, attention, and processing speed were conducted, and change in plasma brain-derived neurotrophic factor (BDNF), Profile of Mood States (POMS), the Healthy People Sleep Quality Index (HPSQI), perceived stress scale (PSS), and salivary cortisol, interleukin (IL)-6, and tumour necrosis factor-α (TNF-α) were assessed at baseline and days 28, 56, and 84.

***Results*:** Supplementation with BDNF Essentials® significantly increased COMPASS tests for memory, attention, and processing speed (all $p < 0.05$). Salivary cortisol was found to be significantly decreased at days 56 and 84 ($p < 0.05$), and IL-6 decreased at day 84 ($P > 0.05$). A reduction in depression-dejection at day 56 and in confusion-bewilderment at day 84, and an improvement in sleep satisfaction at day 56 (all $p < 0.05$) were reported.

***Conclusion*:** BDNF Essentials® improved reaction time in measures of working memory, episodic memory, and attention, reduced biomarkers of stress and inflammation, and improved mood and sleep. BDNF Essentials® has been found to be safe and well-tolerated in adults with self-reported memory complaints.

***Registration Number*:** Registered at Clinicaltrials.gov (NCT04860778).

Keywords: Cognition, BDNF, stress, inflammation, nutraceutical, dietary supplement.

1. INTRODUCTION

A recent survey found 15%-20% of adults over 65 years manifest mild cognitive impairment [1]. Strategies to support cognitive function include exercise, cognitive training, and potential pharmacological agents [2]. However, these interventions are often limited or inconsistent in their efficacy [3-6]. As a result, alternative modalities to maintain cognitive health that are safe and efficacious are gaining consumer interest. Nutraceuticals have demonstrated the ability to attenuate cognitive decline with aging and progression of cognitive diseases [1].

Poor psychological well-being and depression are risk factors associated with self-reported memory complaints [7]. Memory complaints are also associated with poor mental health and quality of life and increased stress in older adults [8]. Self-reported memory complaints have been shown to be associated with subsequent risk of cognitive impairment [9].

There are two types of cognitive functioning often assessed with aging research, *i.e.*, crystallized abilities and fluid abilities. Crystallized abilities refer to general knowledge and skills, such as vocabulary and historical kno-wledge, and remain stable throughout the lifespan [10-12]. Fluid abilities, which refer to the capacity for problem-solving and reasoning, are negatively impacted by age [11, 12]. Many aspects of fluid intelligence peak in the 30s and decline at a rate of approximately -0.02 standard deviations per year [10]. This age-related decline in fluid intelligence may be due to reduced processing speed and working memory [12, 13]. As working memory pertains to the amount of information that individuals can temporarily process [13], improving it can impact many activities of daily life [14]. A meta-analysis found that improvements in working memory corresponded to improvements in generalized everyday functioning across the lifespan [14]. Given the importance of working memory for everyday functioning, the effect of 84-days of supplementation with BDNF Essentials® was examined in a population with self-reported memory complaints.

Brain-derived neurotrophic factor (BDNF) is essential for neuroplasticity and is the most prevalent nerve growth protein shown to stimulate neurogenesis [15]. BDNF influences a variety of functions, including induction of neurogenesis and synapse formation, maintenance and repair of neurons, and supporting overall cognitive function [16]. Several studies have shown an association between BDNF levels and

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neuroplasticity [17-19]. The ingredients found in BDNF Essentials® have been shown to independently improve cognitive function or mood in healthy older adults [20-30].

The objective of this open-label, exploratory study was to investigate the efficacy of the potential synergistic effect of the ingredients that constitute BDNF Essentials® on participants with self-reported memory complaints. Computerized Mental Performance Assessment System (COMPASS) tests were used to assess memory, attention, and processing speed. Changes in plasma BDNF, Profile of Mood States (POMS), Healthy People Sleep Quality Index (HPSQI), perceived stress scale (PSS), salivary cortisol, interleukin (IL)-6, and tumour necrosis factor-α (TNF-α) were assessed. The enrolled study population represented a target group of individuals who would benefit from safe and efficacious nutraceuticals for the support of cognitive function. This transitional stage from normal cognition to cognitive impairment is viewed as an opportune window for interventions to support cognitive improvement [31].

2. MATERIALS AND METHODS

This study was reviewed by the Natural and Non-Prescription Health Product Directorate (NNHPD), Health Canada, and a research ethics board. Notice of authorization was granted on March 19, 2021, by the NNHPD, Ottawa, Ontario. Unconditional approval was granted on March 31, 2021, by the research ethics board (Advarra, Aurora, Ontario). The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and its subsequent amendments. The clinical trial was conducted at KGK Science Inc. (London, ON, Canada) from April–September 2021 and registered at Clinicaltrials.gov (NCT04860778).

2.1. Study Design

Informed consent was obtained from each participant at the screening visit prior to performing any study-related activities. All participants who met the inclusion without meeting any exclusion criteria at screening and/or baseline were required to follow the study protocol. At screening, medical history, concomitant therapies and current health status were reviewed, and the Mini-Mental State Examination (MMSE-2) and Everyday Memory Questionnaire (EMQ) were completed. Information regarding memory and lifestyle was recorded from the participants, including but not limited to age when they noticed changes in their memory and confirmed there had not been changes in personality, as well as sleep, dietary and exercise habits, stress levels, and education. Urine pregnancy tests for female participants of child-bearing potential were administered. Fasting blood samples were collected for the analysis of complete blood count (CBC), electrolytes (sodium, potassium, chloride), hemoglobin (Hb) A1c, glucose, estimated glomerular filtration rate (eGFR), creatinine, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total bilirubin, and a saliva sample collection kit and instructions were dispensed. At baseline (Day 0) and days 28, 56, and 84, data on concomitant therapies and the current health status of participants were reviewed. Saliva samples were collected for cortisol analysis, vitals were measured, and fasting blood samples were collected for BDNF, IL-6, and TNF-α measurements. COMPASS, PSS, POMS, and the HPSQI were administered, and adverse events were recorded.

2.2. Inclusion and Exclusion Criteria

Enrolled participants included males and females 45 years of age and older, who self-reported memory complaints, and had a waist circumference of < 102 cm (40 inches) for males and < 88 cm (35 inches) for females. Participants with a score of ≥ 24 on the MMSE-2 and a score of ≥ 32 on the revised EMQ [32] were eligible. Participants were considered otherwise healthy as determined by laboratory results and medical history as assessed by the Medical Director (MD). Individuals were excluded if they were women who did not meet the study requirements for contraception; had an allergy, sensitivity, or intolerance to the investigational product's active or inactive ingredients; dementia and/or cognitive decline; history of epilepsy, brain injury, or trauma, as assessed by the MD; self-reported colour blindness or confirmation of sleep disorder; had shift work disrupting normal circadian rhythm; intended to or traveled across one or more time zones; used concomitant prescribed, over-the-counter medications, supplements, food and/or drinks; had clinically significant abnormal laboratory results.

2.3. Investigational Product and Directions

BDNF Essentials® is a formulation of Skullcap (*Scutellaria lateriflora*) herb powder, Lion's mane mushroom (*Hericium erinaceus*) powder, Sensoril® (*Withania somnifera*), Ashwagandha root and leaf extract, Bacopa (*Bacopa monnieri*) herb powder, Bilberry fruit extract, cytidine diphosphate choline sodium salt (*Citicoline*), and Sharp-PS® (*Phosphatidylserine*). Participants were instructed to take two capsules with water with or without food twice a day for 84 days.

2.4. Statistical Methods

A total sample size of 20 was estimated to enable the detection of a within-group change in BDNF of 21.6 ng/mL, with a pooled SD of 33.07 ng/mL from baseline to the end of the study [33]. This total sample size was also estimated to enable the detection of within-group changes in COMPASS domains, including speed of attention (24.50 ± 32.30 msec), accuracy of attention (8.87 ± 11.66%), episodic memory (7.34 ± 9.68%), and working memory (3.94 ± 5.16%) [34]. These estimates assumed 5% alpha (one-sided for BDNF, two-sided for COMPASS), 80% power, and a 20% dropout rate.

An efficacy analysis based on the Intention-to-Treat (ITT) population was performed. Variables were tested for normality and log-normality where log-normality distributed variables were analyzed in the logarithmic domain. Appropriate non-parametric tests were used to analyze non-normal variables. All missing values in the ITT were imputed with the most recent previously available value (LOCF, or "last-observation-carried-forward" imputation). No imputation was performed for missing values of safety variables.

2.5. Assessment Tools and Laboratory Methodologies

2.5.1. MMSE-2 Standard Version

The MMSE-2 is a quick 30-point questionnaire, assessing seven areas of cognition, which is widely used for measuring cognitive impairment [35]. The MMSE-2 standard version was administered as a screening tool with a score of ≥ 24 indicating normal cognitive function [36].

2.5.2. EMQ

The EMQ assesses memory failure in everyday life, and a score ≥ 32 identifies those with self-reported memory complaints. The revised EMQ used questions from Factor 1 – Memory and Learning and Factor 3 – Procedure and Monitoring. Each of the questions contained a 9-point Likert scale characterizing the frequency of forgetfulness ranging from 'Not at all in the last six months' to 'More than once a day' [32].

2.5.3 COMPASS

COMPASS, a standardized tool designed to assess cognition (Northumbria University, Newcastle-upon-Tyne, UK), has been shown to be sensitive to nutritional interventions [37, 38]. The pre-programmed cognitive testing system delivers a full set of randomized stimuli for every assessment performed by each participant and comes pre-programmed with word lists, pictures, and names/faces. The tasks were created to assess across major cognitive domains of interest: memory, attention, and processing speed. COMPASS tasks used in the current study included immediate word recall, delayed word recall, picture recognition, word recognition, numeric working memory, Corsi blocks, digit vigilance, Stroop test, choice reaction time, and simple reaction time.

2.5.4. HPSQI

The HPSQI has been previously used to assess sleep quality in a population with occasional sleeplessness and provided an index for the following parameters: sleep efficiency, perceived sleep debt, and sleep difficulty [39]. Participants were asked a total of 19 questions. Five questions captured information regarding a typical 7-day period consisting of 5 weekdays and 2 weekend days. The remaining 14 questions used a 5-point Likert scale ranging from 'Strongly disagree' to 'Strongly agree'.

2.5.5. PSS

The PSS is a 10-item tool that measures the degree to which situations in one's life are considered stressful [40]. Questions were designed to understand respondents' feelings about how unpredictable, uncontrollable, and overloaded their lives are. The scale also includes direct queries to assess the current levels of experienced stress.

2.5.6. POMS

The POMS is a 65-item questionnaire that assesses participant mood [41]. Each item contains a 5-point Likert scale characterizing the possible strengths of a specific feeling (*e.g.*, friendly, sad, helpful) ranging from 'Not at all' to 'Extremely'. Participants were instructed to respond to the questionnaire based on the past week. POMS is a self-reported assessment of mood that is adaptable to capturing transient and fluctuating feelings, or relatively enduring affect states, and contributes to a comprehensive assessment by providing indications of potential mood disturbance. A total mood disturbance score is generated as well as subscales for tension, depression, anger, fatigue, confusion, vigor, and friendliness. The POMS iceberg profile is a visual representation of the 6 scales for the Raw Score. The desirable iceberg shape is characterized by low scores on the tension-anxiety, depression-dejection, anger-hostility, fatigue-inertia, and confusion-bewilderment scales, and high scores on the vigor-activity scale.

2.5.7. Laboratory Methodologies

Blood samples were analyzed for BDNF, IL-6, and TNF-α at screening, and day 0, 28, 56, and 84 at Mount Sinai Hospital (Toronto, Canada). Plasma BDNF blood was collected in EDTA tubes and centrifuged at 1000xg for 10 min. Plasma samples were then diluted 1:2 using the assay buffer and analyzed using EMD Millipore's MILLIPLEX® MAP Human Myokine Magnetic Bead Panel 15-plex kit. The analytical range was 2 to 10,000 pg/mL. Serum IL-6 and TNF-α blood were kept for 30 min to clot, and then centrifuged at 1000xg for 10 min. Serum samples were then analyzed using EMD Millipore's MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel. The analytical range was 3.2 to 2000 pg/mL.

Saliva samples were analyzed for salivary cortisol on the mornings of days 0, 28, 56, and 84 at the London Health Sciences Centre (London, Canada). The saliva was centrifuged for 15 min. Saliva was analyzed using Roche Electrochemiluminescence. The reference range for salivary cortisol collected between 0600h and 1000h was < 24 nmol/L.

3. RESULTS

3.1. Demographics

There was a total of 20 participants in the study, 10 females and 10 males. Participants ranged from 47 to 63 years of age. Most participants were of Western European descent (65%), had a University/Master's degree (60%), regularly exercised (85%), consumed alcohol (75%), and did not smoke tobacco (95%) or use cannabis (95%) (Table **1**).

Table 1. Participant demographic characteristics (n=20).

Demographic Characteristics: ITT Population	
Parameter	**Statistics**
Age (years)	-
Mean (range)	56.15 (47- 63)
Gender [n (%)]	-
Female	10 (50)
Male	10 (50)
Race [n (%)]	-
Western European White	13 (65.0)
Eastern European White	4 (20.0)

(Table 1) contd….

Demographic Characteristics: ITT Population	
Middle Eastern	2 (10.0)
Hispanic or Latino	1 (5.0)
Marital Status [n (%)]	-
Divorced	3 (15.0)
(Married	14 (70.0)
Parameter	**Statistics**
Single	3 (15.0)
Education level [n (%)]	**-**
College diploma	4 (20.0)
High school graduate or GED	3 (15.0)
University/Master's degree	12 (60.0)
Vocational school	1 (5.0)
Employment status [n (%)]	**-**
Full-time	11 (55.0)
Part-time	1 (5.0)
Homemaker	1 (5.0)
Retired	5 (25.0)
Unemployed	2 (10.0)
Alcohol use, Yes [n (%)]	**15 (75.0)**
Tobacco use, No [n (%)]	**19 (95.0)**
Medical marijuana, No [n (%)]	**20 (100.0)**
Recreational marijuana, Yes [n (%)]	**1 (5.0)**
Regular exercise, Yes [n (%)]	**17 (85.0)**
Sleep duration (hours)	**10.82**

Note: n, number; %, percent.

3.2. Participant Disposition

Twenty participants were enrolled in the study, and all were included in the ITT analysis. Participants consumed at least 80% of the investigational product, had no protocol deviations that may have affected the primary outcome, and completed all study visits and procedures (Fig. **1**).

3.3. Plasma BDNF

On day 28 of supplementation, there was a 25% reduction ($p<0.05$) and on day 56, a 12% reduction in BDNF concentration ($p=0.40$) observed. There was a 2.4% increase observed in BDNF concentration on day 84 ($p=0.43$). Although there was no statistically significant mean change in plasma BDNF concentrations in the ITT population after 84 days of supplementation, eight participants had an increase in their plasma BDNF from baseline, and 12 participants had BDNF levels that decreased from their baseline at day 84. Participants whose BDNF increased by 5 ng/mL above their baseline levels were defined as responders. This responder subgroup had differences in their inflammation levels, amount and type of exercise reported, and prevalence of sleep issues at baseline, compared to those who did not respond with an increase in their plasma BDNF (Table **2**). Self-reported information from the responders showed they had a better exercise profile as well at baseline they had higher salivary cortisol, TNF-α, and IL-6. These participants performed similar to the overall group on the COMPASS tests in working memory, episodic memory, attention, and processing speed. However, the responders increased their delayed word recall performance, a finding that was not seen in the overall study population. In responders, picture recognition reaction time ("No") was significantly reduced from baseline at day 84. For both immediate word recall and delayed word recall, there were significant improvements observed on days 56 and 84, compared to baseline (Table **3**).

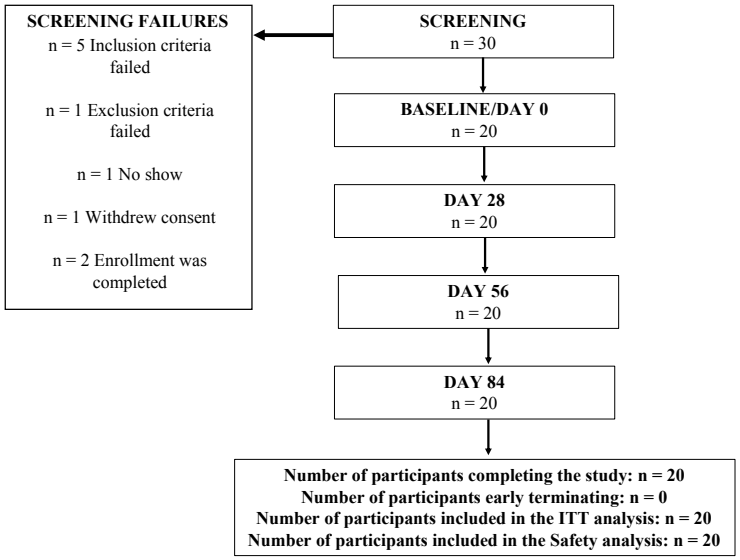

Fig. (1). Participant disposition.

Table 2. Potential variables relating to plasma BDNF levels in responders *vs*. non-responders supplemented with BDNF Essentials®.

Variables at Baseline	Responders (n=8)	Non-responders (n=12)
Age (mean ± SD)	54.6 ± 5.8	57.1 ± 3.4
Gender (% male)	38%	58%
Complete exercise (%)	100%	75%
Complete cardiovascular exercise (%)	75%	33%
EMQ score (mean ± SD)	71.1 ± 19.0	67.5 ± 13.6
BDNF (pg/mL; mean ± SD)	1355.8 ± 1434.7	1652.8 ± 1288.7
PSS score (mean ± SD)	13.0 ± 5.1	15.2 ± 6.2
Salivary cortisol (nmol/L; mean ± SD)	15.1 ± 6.9	11.9 ± 7.3
TNF-α (pg/mL; mean ± SD)	11.0 ± 2.3	9.2 ± 4.0
IL-6 (pg/mL; mean ± SD)	20.1 ± 42.2	9.3 ± 5.9
Difficulty falling asleep (%)	12.5%	33.3%
Difficulty staying asleep through the night (%)	62.5%	58.3%
Refreshing sleep (%)	37.5%	53.3%

Note: n, number; SD, standard deviation ; %, percent.

The results presented below are for the ITT population.

3.4. Memory

BDNF Essentials® formulation improved episodic memory (picture and word recognition), working memory (numeric working memory and Corsi Block tasks), and attention (digit vigilance task and Stroop test) (Table **4**).

Picture recognition reaction time was found to be improved by 13% at days 56 and 84 ($p<0.05$), immediate word recall improved by 15% at day 56 and 41% at day 84 ($p<0.05$), and there was a 15% improvement in delayed word recall at day 56 ($p<0.05$). For numeric working memory, overall, correct and percent correct reaction time improved by 8%, 8%, and 11%, from baseline at day 84, respectively (all $p<0.05$). Both overall and correct reaction times improved by 7% from baseline at day 28, ($p<0.05$), and the percent correct reaction time improved on days 28 and 56 by 11% and 8%, respectively ($p<0.05$). There was a 4% improvement observed in digit vigilance accuracy at day 84 ($p<0.05$). Participants were faster at reacting (overall reaction time) on the Stroop test by 8% on day 56 and 7% on day 84 (all $p<0.05$).

3.5. Processing Speed/Speed of Attention (Choice Reaction and Simple Reaction Time)

There was a 1% decrease in choice reaction time accuracy on day 28 ($p<0.05$), and there were no changes noted on days 56 and 84 (Table **4**). There was no difference observed in simple reaction time.

3.6. Inflammatory Measures

There was a 7% reduction found in IL-6 at day 84 ($p>0.05$; Fig. **2A**). There were no significant changes observed in TNF-α with BDNF Essentials® supplementation (Fig. **2B**).

3.7. Stress and Mood Measures

Salivary cortisol was found to be reduced by 20% and 10% from baseline at days 56 and 84, respectively (all $p<0.05$; Fig. **3A**). There were no changes observed in participants' subjective stress, as assessed by the PSS score throughout the 84-day study (Fig. **3B**).

Participants reported an 18% reduction in confusion-bewilderment at day 84 ($p<0.05$) in the POMS raw score, and a 12% reduction in depression-dejection (percentile) at day 56 ($p<0.05$). The POMS iceberg profile is a visual representation of the six POMS sub-scales. After 84 days of supplementation, participants exhibited the characteristic shape of a positive profile of mood (Fig. **4**).

3.8. Sleep Measures

On day 56, there was an 11% improvement reported in sleep satisfaction ($p<0.05$). There were no significant changes found in sleep efficiency, sleep duration, or sleep-related quality of life during the study.

3.9. Safety Outcomes

There were seven post-emergent adverse events (AEs) reported by five participants. There were two AEs of back pain reported by one participant. There were two AEs of a headache and feeling sick from a second dose of COVID-19 vaccination reported by one participant. The other AEs reported by unique participants were a headache, a cyst, and abnormal liver enzymes. All participants reported resolution of AEs by the end of the study, except for the AE of abnormal liver enzymes, which returned to levels within the normal laboratory range three weeks after the day 84 visit (Supplementary Tables **S1** and **S2**). All AEs were deemed unlikely or not related to BDNF Essentials®.

Table 3. Episodic memory scores at baseline and days 28, 56, and 84 in the responder population with increases in BDNF levels at day 84 (n = 8).

Compass Task	-	Baseline Mean ± SD	Day 28 Mean ± SD *P*-Value*	Day 56 Mean ± SD *P*-Value*	Day 84 Mean ± SD *P*-Value*
Picture recognition	Reaction time (no, msec)	1159.67 ± 413.36	1001.63 ± 206.01 0.49 (w)	1080.14 ± 222.06 0.62 (w)	1023.24 ± 260.61 < 0.05 (w)
Immediate word recall	Total number of points	3.80 ± 1.50	4.0 ± 2.21 0.2	4.47 ± 2.15 < 0.05	4.67 ± 2.5 < 0.05
Delayed word recall	Total number of points	3.02 ± 1.8	3.12 ± 2.3 0.3	3.5 ± 1.7 <0.05	3.59 ± 1.6 <0.05

Abbreviations: msec, milliseconds; SD, standard deviation, %, percent. **p*-values represent scores at days 28, 56, and 84, compared to baseline, and these were generated by the paired *t*-test after untransformed or the paired *t*-test after log-transformed (lp) or the Wilcoxon Signed Rank test (w).

Table 4. COMPASS task scores at baseline and days 28, 56, and 84 (n=20).

Compass Task	-	Baseline Mean ± SD	Day 28 Mean ± SD *P*-Value*	Day 56 Mean ± SD *P*-Value*	Day 84 Mean ± SD *P*-Value*
Episodic Memory					
Picture recognition	Accuracy (overall, %)	96.83 ± 2.95	96.17 ± 3.63 0.5 (w)	97.00 ± 2.84 0.75 (w)	96.33 ± 3.88 0.62
-	Reaction time (no, msec)	1186.51 ± 407.01	1275.57 ± 758.82 0.62 (w)	1031.98 ± 258.35 < 0.05 (w)	1030.50 ± 211.97 < 0.05 (w)
Word recognition	Accuracy (overall, %)	76.50 ± 12.11	75.33 ± 11.21 0.69	78.83 ± 8.60 0.42	78.00 ± 8.19 0.58
Immediate word recall	Total number of points	3.88 ± 1.60	4.08 ± 2.31 0.2	4.47 ± 2.35 < 0.05	5.47 ± 1.97 < 0.05
Delayed word recall	Total number of points	3.12 ± 1.83	3.22 ± 2.23 0.3	3.6 ± 1.8 <0.05	3.80 ± 2.04 0.6
Working Memory					
Numeric working memory	Accuracy (overall, %)	94.05 ± 5.15	95.17 ± 5.37 0.29	93.78 ± 7.53 0.58 (w)	94.61 ± 8.57 0.20 (w)
-	Reaction time (overall, msec)	1177.33 ± 241.18	1089.98 ± 267.83 < 0.05	1115.18 ± 240.16 0.14	1080.04 ± 217.61 < 0.05
-	Reaction time (correct, msec)	1163.28 ± 228.31	1077.96 ± 246.31 < 0.05	1109.79 ± 239.75 0.20	1071.93 ± 212.82 < 0.05
-	Reaction time (yes, msec)	1122.85 ± 246.07	997.38 ± 251.22 < 0.05 (lp)	1033.58 ± 223.08 < 0.05	997.31 ± 206.08 < 0.05
Corsi Blocks	Span score	5.35 ± 1.4	5.57 ± 0.59 0.2	5.67 ± 0.72 0.4	5.47 ± 0.94 0.4
Attention					
Digit vigilance	Accuracy (overall, %)	87.89 ± 9.22	88.22 ± 11.40 0.69	89.00 ± 10.83 0.53	91.11 ± 5.90 < 0.05 (w)
Stroop test	Accuracy (overall, %)	95.50 ± 12.08	93.75 ±14.69 0.48	95.17 ± 14.10 0.63 (w)	99.33 ± 1.37 0.17 (w)

(Table 4) contd….

Compass Task	-	Baseline Mean ± SD	Day 28 Mean ± SD *P*-Value*	Day 56 Mean ± SD *P*-Value*	Day 84 Mean ± SD *P*-Value*
-	Reaction time (overall, msec)	1371.65 ± 309.28	1311.92 ± 353.17 0.80	1266.59 ± 413.9 < 0.05 (w)	1273.67 ± 423.66 < 0.05 (w)
Processing Speed/Speed of Attention					
Choice reaction time	Accuracy (overall, %)	98.9 ± 1.21	97.90 ± 2.2 < 0.05 (w)	98.8 ± 1.36 0.82 (w)	98.40 ± 1.39 0.26 (w)
Simple reaction time	Reaction time (overall, msec)	448.39 ±168.84	635.85 ± 618.45 0.54 (w)	392.83 ± 78.3 0.17 (w)	403.64 ± 134.66 0.10

Note: msec, milliseconds; SD, standard deviation, %, percent. **Note:** * *p*-values represent scores at days 28, 56, and 84, compared to baseline, and these were generated by the paired *t*-test after untransformed or the paired *t*-test after log-transformed (lp) or the Wilcoxon Signed Rank test (w).

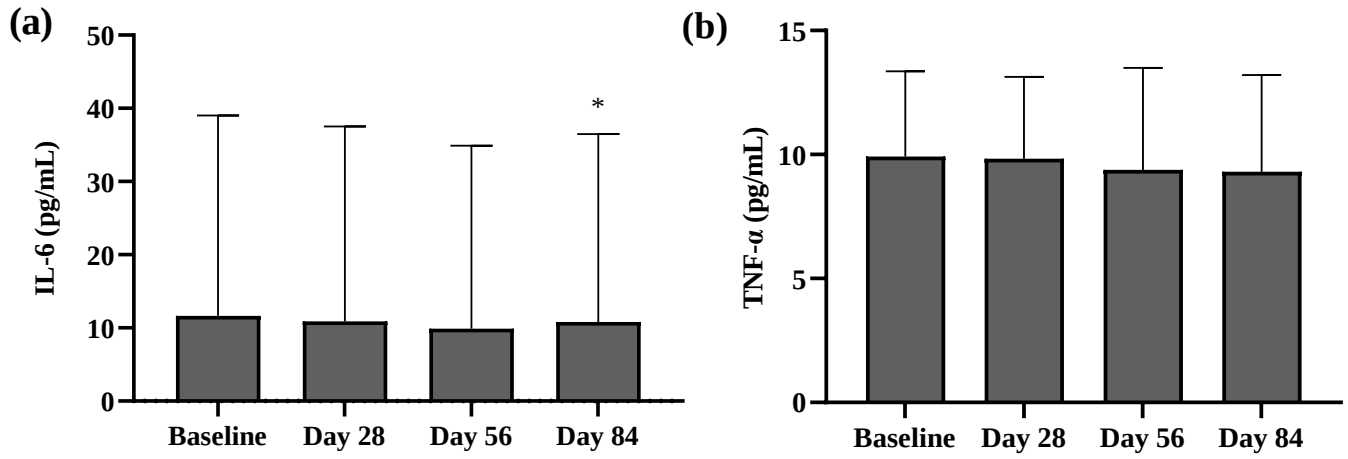

Fig. (2). (**a**) IL-6 and (**b**) TNF-α at baseline and days 28, 56, and 84 after supplementation with BDNF Essentials® (n=20). All values presented are mean ± standard deviation (SD). Changes from the baseline for IL-6 and TNF-α were compared using the paired *t*-test after untransformed or the paired *t*-test after log-transformed or the Wilcoxon Signed Rank test, with $p<0.05$ considered statistically significant.

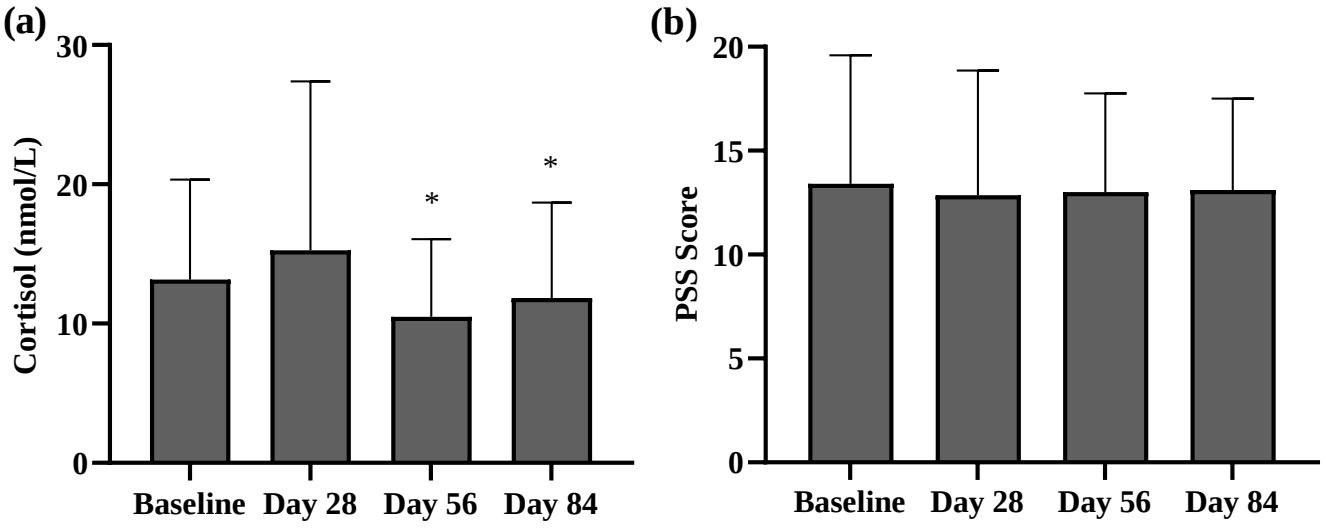

Fig. (3). (a) Cortisol and **(b)** PSS scores at baseline and days 28, 56, and 84 after supplementation with BDNF Essentials® (n=20). All values presented are mean ± SD. Changes from the baseline for cortisol and PSS scores were compared using the paired *t*-test after untransformed or the paired *t*-test after log-transformed or the Wilcoxon Signed Rank test, with $p<0.05$ considered statistically significant. **Note:** * Indicates *p*-value <0.05 significantly different from baseline.

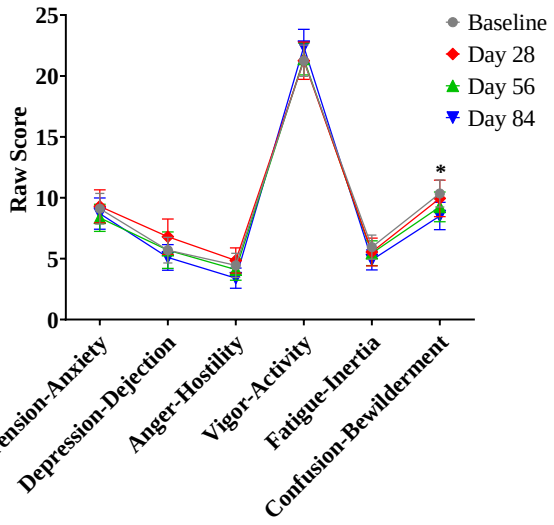

Fig. (4). Profile of Mood States Iceberg profile at baseline and after 28, 56, and 84 days of supplementation with BDNF Essentials®. Data are mean ± standard error of the mean (SEM). Values were compared using the paired *t*-test after untransformed or the paired *t*-test after log-transformed or the Wilcoxon Signed Rank test, with $p<0.05$ considered statistically significant. **Note:** *Indicates significance from baseline at day 84. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

4. DISCUSSION

The population enrolled in this study included healthy individuals with normal cognition and self-reporting issues with memory but no pathologies.

Supplementation with BDNF Essentials® for 84 days significantly increased scores on COMPASS tests for memory, attention, and processing speed. Overall, there were improvements in reaction time for domains of episodic and working memory, and attention.

Episodic memory plays an important role in everyday functioning, as this is the memory relied upon to recall information, such as where a car is parked, where one left their keys, or the location of the doctor's office. BDNF Essentials® supplementation resulted in increased performance on working memory and episodic memory tests. Although accuracy was not impacted by supplementation, there were improvements in reaction time. Actions and responses, both for decisions and movements, performed faster are typically coupled with diminished accuracy [42, 43]; however, this was not observed with this supplement. Further, accuracy may not have improved as baseline accuracy was high for most measures (*e.g.*, picture recognition 97%; numeric working memory 94%; Stroop test 95%; and choice reaction time 99%).

BDNF Essentials® increased attentional performance with accuracy in the digit vigilance task and faster reaction times during the Stroop test. The increase in accuracy of digit vigilance, a number-matching task, was the only significant increase in accuracy observed. The digit vigilance test assessed both sustained attention and psychomotor speed [44]; thus, the increased accuracy with no associated change in speed in this task suggests that BDNF Essentials® may augment attentional performance without negatively impacting speed. In all reaction time measures during the Stroop test, supplementation with BDNF Essentials® significantly increased performance relative to baseline. Selective attention, which is the ability to focus on relevant task information and disregard extraneous information, is assessed by the Stroop test and it has been shown to decline with age [12]. Bacopa supplementation for 12 weeks in healthy older adults was previously shown to increase Stroop test performance compared to placebo [45], whereas phosphatidylserine supplementation for the same duration had no effect on the Stroop test [46]. In older adults (50-85 years) with subjective memory complaints, there was reduced brain activity reported corresponding to task-related attention compared to adults without subjective memory complaints [47]. Therefore, it is possible to suggest that the increased attention observed with BDNF Essentials® may alleviate some of the memory-related issues that occur with aging.

Although processing speed has been shown to decline with age [11, 12], the direct test of processing speed or speed of attention within COMPASS was not affected by supplementation with BDNF Essentials®. Bacopa supplementation improved the speed of attention by reducing choice reaction time in healthy participants [48]. In the current study, there were, however, improvements in reaction time, as found in several of the other cognitive test assessments. These data indicate that while BDNF Essentials® did not result in improvements in reaction time tasks that require little-to-no cognitive processing, such as the simple- and choice-reaction time tests, there were improvements in reaction time in tasks that required more complex cognitive processing, such as the Stroop test and numeric working memory tests. In these more complex tasks, participants had to perceive the stimulus, process the visual information, and then generate an appropriate response [11]. Therefore, supplementation with BDNF Essentials® may have augmented processing times for making decisions [11].

It is interesting to note that there was no speed-accuracy trade-off observed in this study. Typically, movements conducted at a faster speed are associated with lower accuracy [42]. Although this phenomenon is found in motor control, it has implications for decision-making. Decisions made faster are less accurate and decisions made slower are more accurate [43]. However, with BDNF Essentials®, not only was overall reaction time significantly increased for working memory, episodic memory, and attention tests, reaction time was faster when making the correct decisions. The potential to augment neural processing speed while not sacrificing accuracy is certainly of value in this population with memory complaints.

Plasma BDNF remained unchanged perhaps due to the large inter- and intra-participant variability observed. Similar variability has been previously reported even after 12-month longer supplementation [49]. BDNF is an important neurotrophin that plays a crucial role during age-related synaptic loss [50, 51] and cognitive decline. Various confounders, such as age, cognitive state, lifestyle, and disease state, have been reported to cause variability of BDNF [49, 51-53]. Over 100 polymorphisms in the BDNF gene exist, resulting

in variations in BDNF levels. Approximately 20-30% of the worldwide population have a heterozygous (val66met) polymorphism [52, 54, 55]. Memory and executive function have been demonstrated to be associated with the BDNF val66met polymorphism [54], where the heterozygous polymorphism is associated with significantly worse performance on a working memory task [56]. The high variability of BDNF levels in healthy subjects is thought to be associated with the highly regulated expression of BDNF [17]. Changes in expression have been reported to occur during both normal and pathological aging [17].

Normal aging is associated with increased levels of pro-inflammatory markers and cytokines causing chronic inflammation, termed "inflamm-aging" and corresponds to an increased risk of morbidity and mortality [57]. In healthy adults, elevated levels of IL-6 and TNF-α are biomarkers for increased risk of cognitive decline [57], and the reductions in IL-6 observed in this study are consistent with other reports [58]. IL-6 is inversely correlated with memory performance, especially in women [59]. A supplement containing *Bacopa monnieri*, *Hippophae rhamnoides*, and *Dioscorea bulbifera,* was reported to significantly decrease IL-6 and TNF-α levels in healthy adults [60]. Ashwagandha prevented cognitive decline from systemic- and neuro-inflammation in pre-clinical models [61]. It is possible to suggest that improvement in COMPASS tasks with BDNF Essentials® may be attributed to reduced systemic inflammation in adults self-reporting memory complaints.

Elevated stress contributes to cognitive decline and memory impairments [62]. Salivary cortisol reflects circulating levels of cortisol [63, 64], and elevated cortisol concentrations are associated with hippocampus-dependent memory deficits in older adults [62]. BDNF Essentials® decreased salivary cortisol. However, the decrease in salivary cortisol was not associated with reductions in the PSS. The mean PSS score in this population was 13.4 at baseline, which is higher than the United States population norms in 1994 for this age group (55-65 years, PSS = 11.9) [65], and the Swedish population norms reported in 2013 (55-79 years, PSS = 12.9) [66]. This study was conducted during the COVID-19 pandemic (May-September 2021); therefore, elevated stress levels were expected [67]. The current population from Ontario had a lower PSS score than adults in Alberta who joined the Text4Hope program (60 years and over, PSS = 16.65) during the COVID-19 pandemic [67]. Ninety days of supplementation with ashwagandha root reduced both serum cortisol and perceived stress in healthy young and older adults with self-reported elevated stress levels (20-55 years, PSS = 19.5) [68]. It is possible to suggest that BDNF Essentials® may be beneficial in reducing subjective stress scores in populations self-reporting high-stress levels [68].

There were improvements in mood with BDNF Essentials®, with significant reductions in confusion-bewilderment and depression-dejection at days 84 and 56, respectively. Confusion-bewilderment is a mood reflecting anxiety from bewilderment and cognitive inefficiency and depression-dejection is associated with unhappiness, guilt, isolation, worthlessness, and hopelessness [69]. One of the hallmark symptoms of subjective cognitive decline is increasingly frequent feelings of confusion within the last 12 months [70]. Older adults with subjective memory complaints are often associated with depression symptoms or a diagnosis of depression [71]. Two weeks of skullcap supplementation in healthy adults (19-66 years) has been shown to improve mood scores on POMS [26].

Ingredients in BDNF Essentials®, such as ashwagandha, have been shown to be beneficial for sleep [68]; however, bacopa and skullcap have not [26, 72]. The typical sleep duration for middle-aged and older-adult populations is 9.1 and 8.1 hours, respectively [73]. The population studied had a relatively long sleep duration (10.82 hours) prior to supplementation; hence, improvements in sleep would have been more challenging to demonstrate in such a context.

The subgroup of responders reported exercising on a weekly basis, with 75% completing cardiovascular exercise. Given the positive effects of aerobic exercise on BDNF levels, it is possible to suggest that the response to BDNF Essentials® acted synergistically with exercise in the responders. Self-reported baseline information from this group suggested that their consistent commitment to aerobic exercise may have been of value in the elevation of their plasma BDNF.

Also, at baseline, the responder population had a mean score of 13 on the PSS (a score of 0-13 is considered low stress) [74, 75], and continued to have reductions in their PSS scores over the 84-day supplementation period, with a 23% reduction in PSS score at day 84, which remained within the low-stress range. Responders had higher levels of salivary cortisol at baseline compared to non-responders. Salivary cortisol has a diurnal pattern, which is characterized by high levels upon waking, with a peak typically occurring within 30 minutes of waking and a nadir occurring in the afternoon [76]. Saliva was collected at a single time point within 30 minutes of waking, and hence, it was not possible to gauge the time of the day when the profile nadir occurred.

A higher number of memory complaints were reported by the responders compared to non-responders (71.1 *vs*. 67.5 EMQ score). Previous studies have shown that lower BDNF levels are associated with lower cognitive test scores and mild cognitive impairment [51]. As the study population did not have any pathologies related to memory, this may suggest that BDNF Essentials® was most beneficial to those with a higher number of self-reported memory complaints.

Mean baseline IL-6 concentrations were higher in the responders, and there were 5.4% and 21% reductions on days 28 and 56, and a 22.5% increase on day 84 in the population. It is possible that BDNF Essentials® may have been efficacious in reducing IL-6 levels in the responder population, which may be related to an increase in BDNF over the 84-day study period. Self-reported difficulty in sleeping and the higher number of memory complaints by the responders may suggest that these participants may have greater stress in their lives. Interestingly, the PSS scores of the responders were at borderline high levels, albeit within the normal range. Previously, other studies have reported an integrative role between BDNF and cortisol, suggesting that increases in the latter may be involved in the progression to stress-related disorders [77]. It is possible that BDNF may play a role in early targeted prevention of such disorders.

As the data reveal, improving cognition outcomes and biomarkers is certainly challenging when investigating healthy participants as it leaves little room for improvement. Baseline inflammation and PSS scores in this population may have played a role in the response to BDNF Essentials® and are limitations that require further investigation. The results of this study are promising and certainly allow for an addition to the evidence on BDNF Essentials®. However, as this was an open-label exploratory study, it is important that the BDNF Essentials® formulation be examined in a more rigorous randomized clinical study to confirm the current results. This study did not select a population based on activity level; however, future studies investigating the influence of exercise on cognition in an exercise model may be of value. This study investigated the efficacy of the formulation. Thus, a placebo group was not factored into the study design. The intent being to provide a foundation for future longer-term randomized double-blind placebo-controlled studies, which may investigate the effect of BDNF Essentials® in certain sub-populations (*e.g.*, those engaging in cardiovascular exercise) as they may respond more favorably to supplementation. Certainly, the exploratory design and small sample size accentuated the high interindividual variation between these markers and are limitations that did not allow for any further investigation of the results. Long-term placebo-controlled studies with larger sample sizes are required for confirmation of the role of BDNF Essentials® and its effect on those with higher PSS scores.

Future studies on BDNF Essentials® in populations with cut-offs at a higher EMQ and greater subjective memory complaints, as well as the relationship between IL-6 and the BNDF cortisol ratios at baseline, are certainly warranted [78].

CONCLUSION

The results of this exploratory study showed that supplementing with BDNF Essentials® for 84 days significantly improved reaction time in measures of working memory, episodic memory, and attention in a single cohort of healthy participants. There was no speed-accuracy trade-off observed in this study as improvements in reaction time were observed while a high level of accuracy was maintained. The improvements in reaction time observed in more complex tasks were not carried over to simple tasks, such as the choice- and simple-reaction time tasks. Furthermore, participants reported a reduction in their confusion and bewilderment and depression-dejection scores, resulting in a better profile of their mood.

It is interesting that improvements were observed in the absence of changes to plasma BDNF levels. Supplementation with BDNF Essentials® led to reductions in salivary cortisol and IL-6 levels, which are the biomarkers of stress and inflammation. Aerobic exercise, stress levels, and degree of self-reported memory complaints appeared to influence the response to BDNF Essentials®. Based on the literature and the findings of the current study, responses to BDNF Essentials® were influenced by a variety of factors beyond baseline BDNF levels. Further research is certainly warranted to confirm the results of this study. BDNF Essentials® was found to be safe and well-tolerated in this population of adults with self-reported memory complaints during the 84-day study period.

LIST OF ABBREVIATIONS

AE	=	Adverse event
ALP	=	Alkaline phosphatase
ALT	=	Alanine transaminase
AST	=	Aspartate transaminase
BDNF	=	Brain-derived neurotrophic factor
CBC	=	Complete blood count
COMPASS	=	Computerized Mental Performance Assessment System
eGFR	=	Estimated glomerular filtration rate
EMQ	=	Everyday Memory Questionnaire
HPSQI	=	Healthy People Sleep Quality Index
IL-6	=	Interleukin-6
ITT	=	Intent-to-Treat
LOCF	=	Last-observation-carried-forward
MMSE-2	=	Mini-Mental State Examination
NNHPD	=	Natural and Non-Prescription Health Product Directorate
POMS	=	Profile of Mood States
PSS	=	Perceived stress scale
TNF-α	=	Tumour necrosis factor

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Institutional Review Board (IRB) Services (pro00050873, Advara, Aurora, Ontario; approved on 31st March, 2021).

HUMAN AND ANIMAL RIGHTS

No animals were used in this study. All procedures involving human participants were conducted in accordance with the Declaration of Helsinki.

CONSENT FOR PUBLICATION

Informed consent was obtained from all subjects involved in the study.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated and/or analyzed during the current study will be available from the corresponding author upon reasonable request.

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STANDARDS OF REPORTING

CONSORT guidelines and methodology were followed.

CONFLICT OF INTEREST

D.H. is an employee of Researched Nutritionals; LLC. N.G., E.D.L., M.E., and D.C.C. have no financial interests in or conflict with the subject matter or materials discussed.

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SUPPLEMENTARY MATERIAL

Supplementary Table **1**. CONSORT 2010 checklist of information to include when reporting a randomized trial.

Supplementary Table **S1**. Change in hematology parameters from pre-supplementation to day 84 in the ITT population (n = 20).

Supplementary Table **S2**. Change in clinical chemistry parameters from pre-supplementation to day 84 in the ITT population (n = 20).

REFERENCES

[1] Singh, P.; Sivanandam, T.M.; Konar, A.; Thakur, M.K. Role of nutraceuticals in cognition during aging and related disorders. *Neurochem. Int.,* **2021**, *143*, 104928. http://dx.doi.org/10.1016/j.neuint.2020.104928 PMID: 33285273

[2] Różyk-Myrta, A.; Wolińska, A.; Kołat, E.; Różyk-Myrta, A. Guidelines for prevention and treatment of cognitive impairment in the elderly. *Med. Sci. Monit.,* **2015**, *21*, 585-597. http://dx.doi.org/10.12659/MSM.892542 PMID: 25708246

[3] Naqvi, R.; Liberman, D.; Rosenberg, J.; Alston, J.; Straus, S. Preventing cognitive decline in healthy older adults. *CMAJ,* **2013**, *185*(10), 881-885. http://dx.doi.org/10.1503/cmaj.121448 PMID: 23589432

[4] Rebok, G.W.; Ball, K.; Guey, L.T.; Jones, R.N.; Kim, H.Y.; King, J.W.; Marsiske, M.; Morris, J.N.; Tennstedt, S.L.; Unverzagt, F.W.; Willis, S.L. Ten-year effects of the advanced cognitive training for independent and vital elderly cognitive training trial on cognition and everyday functioning in older adults. *J. Am. Geriatr. Soc.,* **2014**, *62*(1), 16-24. http://dx.doi.org/10.1111/jgs.12607 PMID: 24417410

[5] Cassilhas, R.C.; Viana, V.A.; Grassmann, V.; Santos, R.T.; Santos, R.F.; Tufik, S.; Mello, M.T. The impact of resistance exercise on the cognitive function of the elderly. *Med. Sci. Sports. Exerc.,* **2007**, *39*(8), 1401-1407. http://dx.doi.org/10.1249/mss.0b013e318060111f

[6] Carlessi, A.S.; Borba, L.A.; Zugno, A.I.; Quevedo, J.; Réus, G.Z. Gut microbiota-brain axis in depression: The role of neuroinflammation. *Eur. J. Neurosci.,* **2019**, *53*(1), 222-235. PMID: 31785168

[7] Benito-León, J.; Mitchell, A.J.; Vega, S.; Bermejo-Pareja, F. A population-based study of cognitive function in older people with subjective memory complaints. *J. Alzheimers Dis.,* **2010**, *22*(1), 159-170. http://dx.doi.org/10.3233/JAD-2010-100972 PMID: 20847410

[8] Haag, F.; Adriouch, S.; Braß, A.; Jung, C.; Möller, S.; Scheuplein, F.; Bannas, P.; Seman, M.; Koch-Nolte, F. Extracellular NAD and ATP: Partners in immune cell modulation. *Purinergic Signal.,* **2007**, *3*(1-2), 71-81. http://dx.doi.org/10.1007/s11302-006-9038-7 PMID: 18404420

[9] Kryscio, R.J.; Abner, E.L.; Cooper, G.E.; Fardo, D.W.; Jicha, G.A.; Nelson, P.T.; Smith, C.D.; Van Eldik, L.J.; Wan, L.; Schmitt, F.A. Self-reported memory complaints: Implications from a longitudinal cohort with autopsies. *Neurology,* **2014**, *83*(15), 1359-1365. http://dx.doi.org/10.1212/WNL.0000000000000856 PMID: 25253756

[10] Salthouse, T. Consequences of age-related cognitive declines. *Annu. Rev. Psychol.,* **2012**, *63*(1), 201-226. http://dx.doi.org/10.1146/annurev-psych-120710-100328 PMID: 21740223

[11] Murman, D. The impact of age on cognition. *Semin. Hear.,* **2015**, *36*(3), 111-121. http://dx.doi.org/10.1055/s-0035-1555115 PMID: 27516712

[12] Harada, C.N.; Natelson, L.M.C.; Triebel, K.L. Normal cognitive aging. *Clin. Geriatr. Med.,* **2013**, *29*(4), 737-752. http://dx.doi.org/10.1016/j.cger.2013.07.002 PMID: 24094294

[13] Teixeira-Santos, A.C.; Moreira, C.S.; Magalhães, R.; Magalhães, C.; Pereira, D.R.; Leite, J.; Carvalho, S.; Sampaio, A. Reviewing working memory training gains in healthy older adults: A meta-analytic review of transfer for cognitive outcomes. *Neurosci. Biobehav. Rev.,* **2019**, *103*, 163-177. http://dx.doi.org/10.1016/j.neubiorev.2019.05.009 PMID: 31100297

[14] Spencer-Smith, M.; Klingberg, T. Benefits of a working memory training program for inattention in daily life: a systematic review and meta-analysis. *PLoS One,* **2015**, *10*(3), e0119522. http://dx.doi.org/10.1371/journal.pone.0119522 PMID: 25793607

[15] Bathina, S.; Das, U.N. Brain-derived neurotrophic factor and its clinical implications. *Arch. Med. Sci.,* **2015**, *6*(6), 1164-1178. http://dx.doi.org/10.5114/aoms.2015.56342 PMID: 26788077

[16] Andero, R.; Choi, D.C.; Ressler, K.J. BDNF-TrkB receptor regulation of distributed adult neural plasticity, memory formation, and psychiatric disorders. *Prog. Mol. Biol. Transl. Sci.,* **2014**, *122*, 169-192. http://dx.doi.org/10.1016/B978-0-12-420170-5.00006-4 PMID: 24484701

[17] Miranda, M.; Morici, J.F.; Zanoni, M.B.; Bekinschtein, P. Brain-derived neurotrophic factor: A key molecule for memory in the healthy and the pathological brain. *Front. Cell. Neurosci.,* **2019**, *13*, 363. http://dx.doi.org/10.3389/fncel.2019.00363 PMID: 31440144

[18] Phillips, C. Brain-derived neurotrophic factor, depression, and physical activity: Making the neuroplastic connection. *Neural Plast.,* **2017**, *2017*, 7260130. http://dx.doi.org/10.1155/2017/7260130

[19] Sangiovanni, E.; Brivio, P.; Dell'Agli, M.; Calabrese, F. Botanicals as modulators of neuroplasticity: Focus on BDNF. *Neural Plast.,* **2017**, *2017*, 5965371. http://dx.doi.org/10.1155/2017/5965371

[20] Peth-Nui, T.; Wattanathorn, J.; Muchimapura, S.; Tong-Un, T.; Piyavhatkul, N.; Rangseekajee, P.; Ingkaninan, K.; Vittaya-areekul, S. Effects of 12-Week *Bacopa monnieri* consumption on attention, cognitive processing, working memory, and functions of both cholinergic and monoaminergic systems in healthy elderly volunteers. *Evid. Based Complement. Alternat. Med.,* **2012**, *2012*, 1-10. http://dx.doi.org/10.1155/2012/606424 PMID: 23320031

[21] Saitsu, Y.; Nishide, A.; Kikushima, K.; Shimizu, K.; Ohnuki, K. Improvement of cognitive functions by oral intake of Hericium erinaceus. *Biomed. Res.,* **2019**, *40*(4), 125-131. http://dx.doi.org/10.2220/biomedres.40.125 PMID: 31413233

[22] Cotroneo, A.M.; Castagna, A.; Putignano, S.; Lacava, R.; Fantò, F.; Monteleone, F.; Rocca, F.; Malara, A.; Gareri, P. Effectiveness and safety of citicoline in mild vascular cognitive impairment: The IDEALE study. *Clin. Interv. Aging,* **2013**, *8*, 131-137. PMID: 23403474

[23] Kato-Kataoka, A.; Sakai, M.; Ebina, R.; Nonaka, C.; Asano, T.; Miyamori, T. Soybean-derived phosphatidylserine improves memory function of the elderly Japanese subjects with memory complaints. *J. Clin. Biochem. Nutr.,* **2010**, *47*(3), 246-255.

http://dx.doi.org/10.3164/jcbn.10-62 PMID: 21103034

[24] Pingali, U.; Pilli, R.; Fatima, N. Effect of standardized aqueous extract of Withania somniferaon tests of cognitive and psychomotor performance in healthy human participants. *Pharmacognosy Res.,* **2014**, *6*(1), 12-18. http://dx.doi.org/10.4103/0974-8490.122912 PMID: 24497737

[25] Choudhary, D.; Bhattacharyya, S.; Bose, S. Efficacy and safety of ashwagandha (Withania somnifera (L.) Dunal) root extract in improving memory and cognitive functions. *J. Diet. Suppl.,* **2017**, *14*(6), 599-612. http://dx.doi.org/10.1080/19390211.2017.1284970 PMID: 28471731

[26] Brock, C.; Whitehouse, J.; Tewfik, I.; Towell, T. American skullcap (Scutellaria lateriflora): A randomised, double-blind placebo-controlled crossover study of its effects on mood in healthy volunteers. *Phytother. Res.,* **2014**, *28*(5), 692-698. http://dx.doi.org/10.1002/ptr.5044 PMID: 23878109

[27] Mori, K.; Inatomi, S.; Ouchi, K.; Azumi, Y.; Tuchida, T. Improving effects of the mushroom Yamabushitake (*Hericium erinaceus*) on mild cognitive impairment: A double-blind placebo-controlled clinical trial. *Phytother. Res.,* **2009**, *23*(3), 367-372. http://dx.doi.org/10.1002/ptr.2634 PMID: 18844328

[28] EghbaliFeriz, S.; Taleghani, A.; Tayarani-Najaran, Z. Central nervous system diseases and Scutellaria: A review of current mechanism studies. *Biomed. Pharmacother.,* **2018**, *102*, 185-195. http://dx.doi.org/10.1016/j.biopha.2018.03.021 PMID: 29554597

[29] Kidd, P.M. A review of nutrients and botanicals in the integrative management of cognitive dysfunction. *Altern Med Rev.,* **1999**, *4*(3), 144-161.

[30] Pase, M.P.; Kean, J.; Sarris, J.; Neale, C.; Scholey, A.B.; Stough, C. The cognitive-enhancing effects of Bacopa monnieri: a systematic review of randomized, controlled human clinical trials. *J. Altern. Complement. Med.,* **2012**, *18*(7), 647-652. http://dx.doi.org/10.1089/acm.2011.0367 PMID: 22747190

[31] Anderson, N.D. State of the science on mild cognitive impairment (MCI). *CNS Spectr.,* **2019**, *24*(1), 78-87. http://dx.doi.org/10.1017/S1092852918001347 PMID: 30651152

[32] Calabria, M.; Manenti, R.; Rosini, S.; Zanetti, O.; Miniussi, C.; Cotelli, M. Objective and subjective memory impairment in elderly adults: A revised version of the Everyday Memory Questionnaire. *Aging Clin. Exp. Res.,* **2011**, *23*(1), 67-73. http://dx.doi.org/10.1007/BF03324954 PMID: 21499021

[33] Bowtell, J.L.; Aboo-Bakkar, Z.; Conway, M.E.; Adlam, A.R.; Fulford, J. Enhanced task-related brain activation and resting perfusion in healthy older adults after chronic blueberry supplementation. *Appl Physiol Nutr Metab.,* **2017**, *42*(7), 773-779.

[34] Wightman, E.L.; Jackson, P.A.; Forster, J.; Khan, J.; Wiebe, J.C.; Gericke, N.; Kennedy, D.O. Acute effects of a polyphenol-rich leaf extract of *Mangifera indica* L. (Zynamite) on cognitive function in healthy adults: A double-blind, placebo-controlled crossover study. *Nutrients,* **2020**, *12*(8), 2194. http://dx.doi.org/10.3390/nu12082194 PMID: 32717999

[35] Folstein, M.F.; Folstein, S.E.; White, T.; Messer, M.A. *MMSE-2: Mini-mental state examination*; Psychological Assessment Resources, **2010**.

[36] Ralat, S.I. Using the MMSE-2 to measure cognitive deterioration in a sample of psychiatric patients living in Puerto Rico. *Int. J. Environ. Res. Public Health,* **2021**, *18*(4), 1694. http://dx.doi.org/10.3390/ijerph18041694 PMID: 33578762

[37] Wightman, E.L.; Haskell-Ramsay, C.F.; Thompson, K.G.; Blackwell, J.R.; Winyard, P.G.; Forster, J.; Jones, A.M.; Kennedy, D.O. Dietary nitrate modulates cerebral blood flow parameters and cognitive performance in humans: A double-blind, placebo-controlled, crossover investigation. *Physiol. Behav.,* **2015**, *149*, 149-158. http://dx.doi.org/10.1016/j.physbeh.2015.05.035 PMID: 26037632

[38] Steiner, G.Z.; Yeung, A.; Liu, J.X.; Camfield, D.A.; Blasio, F.M.; Pipingas, A.; Scholey, A.B.; Stough, C.; Chang, D.H. The effect of Sailuotong (SLT) on neurocognitive and cardiovascular function in healthy adults: A randomised, double-blind, placebo controlled crossover pilot trial. *BMC Complement. Altern. Med.,* **2015**, *16*(1), 15. http://dx.doi.org/10.1186/s12906-016-0989-0 PMID: 26762282

[39] Moulin, M.; Lewis, E.D.; Crowley, D.C.; Langston, J.; Evans, M. A randomized, double-blind, placebo-controlled, cross-over pilot study to investigate the efficacy of Rest-ZZZ formula in healthy participants with occasional sleeplessness. *Sleep Biol. Rhythms,* **2022**, 1-10.

[40] Cohen, S. Perceived stress in a probability sample of the United States. Spacapan, S.O. *The social psychology of health,* Sage Publications, Inc**1988**, 31-67.

[41] McNair, D.; Lorr, M.; Droppleman, L. *Manual for the profile of mood states (POMS)*; Educational and Industrial Testing Service: San Diego, **1971**.

[42] Fitts, P.M. The information capacity of the human motor system in controlling the amplitude of movement. *J. Exp. Psychol.,* **1954**, *47*(6), 381-391. http://dx.doi.org/10.1037/h0055392 PMID: 13174710

[43] Standage, D.; Blohm, G.; Dorris, M.C. On the neural implementation of the speed-accuracy trade-off. *Front. Neurosci.,* **2014**, *8*, 236. http://dx.doi.org/10.3389/fnins.2014.00236 PMID: 25165430

[44] Kelland, D.Z.; Lewis, R.F. The digit vigilance test: Reliability, validity, and sensitivity to diazepam. *Arch. Clin. Neuropsychol.,* **1996**, *11*(4), 339-344. http://dx.doi.org/10.1093/arclin/11.4.339 PMID: 14588938

[45] Calabrese, C.; Gregory, W.L.; Leo, M.; Kraemer, D.; Bone, K.; Oken, B. Effects of a standardized Bacopa monnieri extract on cognitive performance, anxiety, and depression in the elderly: A randomized, double-blind, placebo-controlled trial. *J. Altern. Complement. Med.,* **2008**, *14*(6), 707-713. http://dx.doi.org/10.1089/acm.2008.0018 PMID: 18611150

[46] Jorissen, B.L.; Brouns, F.; Van Boxtel, M.P.J.; Ponds, R.W.H.M.; Verhey, F.R.J.; Jolles, J.; Riedel, W.J. The influence of soy-derived phosphatidylserine on cognition in age-associated memory impairment. *Nutr. Neurosci.,* **2001**, *4*(2), 121-134. http://dx.doi.org/10.1080/1028415X.2001.11747356 PMID: 11842880

[47] Hayes, J.M.; Tang, L.; Viviano, R.P.; van Rooden, S.; Ofen, N.; Damoiseaux, J.S. Subjective memory complaints are associated with brain activation supporting successful memory encoding. *Neurobiol. Aging,* **2017**, *60*, 71-80. http://dx.doi.org/10.1016/j.neurobiolaging.2017.08.015 PMID: 28923533

[48] Kongkeaw, C.; Dilokthornsakul, P.; Thanarangsarit, P.; Limpeanchob, N.; Norman, S.C. Meta-analysis of randomized controlled trials on cognitive effects of Bacopa monnieri extract. *J. Ethnopharmacol.,* **2014**, *151*(1), 528-535. http://dx.doi.org/10.1016/j.jep.2013.11.008 PMID: 24252493

[49] Naegelin, Y.; Dingsdale, H.; Säuberli, K.; Schädelin, S.; Kappos, L.; Barde, Y.-A. Measuring and validating the levels of brain-derived neurotrophic factor in human serum. *eNeuro,* **2018**, *5*(2), ENEURO.0419-17.2018. http://dx.doi.org/10.1523/ENEURO.0419-17.2018

[50] Molinari, C.; Morsanuto, V.; Ruga, S.; Notte, F.; Farghali, M.; Galla, R.; Uberti, F. The role of BDNF on aging-modulation markers. *Brain Sci.,* **2020**, *10*(5), 285. http://dx.doi.org/10.3390/brainsci10050285 PMID: 32397504

[51] Shimada, H.; Makizako, H.; Doi, T.; Yoshida, D.; Tsutsumimoto, K.; Anan, Y.; Uemura, K.; Lee, S.; Park, H.; Suzuki, T.; Large, A. A large, cross-sectional observational study of serum BDNF, cognitive function, and mild cognitive impairment in the elderly. *Front. Aging Neurosci.,* **2014**, *6*, 69.

[52] Rahmani, M.; Rahmani, F.; Rezaei, N. The brain-derived neurotrophic factor: missing link between sleep deprivation, insomnia, and depression. *Neurochem. Res.,* **2020**, *45*(2), 221-231. http://dx.doi.org/10.1007/s11064-019-02914-1 PMID: 31782101

[53] Komulainen, P.; Pedersen, M.; Hänninen, T.; Bruunsgaard, H.; Lakka, T.A.; Kivipelto, M.; Hassinen, M.; Rauramaa, T.H.; Pedersen, B.K.; Rauramaa, R. BDNF is a novel marker of cognitive function in ageing women: The DR's EXTRA Study. *Neurobiol. Learn. Mem.,* **2008**, *90*(4), 596-603. http://dx.doi.org/10.1016/j.nlm.2008.07.014 PMID: 18707012

[54] Toh, Y.L.; Ng, T.; Tan, M.; Tan, A.; Chan, A. Impact of brain-derived neurotrophic factor genetic polymorphism on cognition: A systematic review. *Brain Behav.,* **2018**, *8*(7), e01009.

http://dx.doi.org/10.1002/brb3.1009 PMID: 29858545

[55] Egan, M.F.; Kojima, M.; Callicott, J.H.; Goldberg, T.E.; Kolachana, B.S.; Bertolino, A.; Zaitsev, E.; Gold, B.; Goldman, D.; Dean, M.; Lu, B.; Weinberger, D.R. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell,* **2003**, *112*(2), 257-269. http://dx.doi.org/10.1016/S0092-8674(03)00035-7 PMID: 12553913

[56] Alfimova, M.V.; Korovaitseva, G.I.; Lezheiko, T.V.; Golimbet, V.E. Effect of BDNF Val66Met polymorphism on normal variability of executive functions. *Bull. Exp. Biol. Med.,* **2012**, *152*(5), 606-609. http://dx.doi.org/10.1007/s10517-012-1587-x PMID: 22803145

[57] Michaud, M.; Balardy, L.; Moulis, G.; Gaudin, C.; Peyrot, C.; Vellas, B.; Cesari, M.; Nourhashemi, F. Proinflammatory cytokines, aging, and age-related diseases. *J. Am. Med. Dir. Assoc.,* **2013**, *14*(12), 877-882. http://dx.doi.org/10.1016/j.jamda.2013.05.009 PMID: 23792036

[58] Stenfors, C.U.D.; Jonsdottir, I.H.; Magnusson Hanson, L.L.; Theorell, T. Associations between systemic pro-inflammatory markers, cognitive function and cognitive complaints in a population-based sample of working adults. *J. Psychosom. Res.,* **2017**, *96*, 49-59. http://dx.doi.org/10.1016/j.jpsychores.2017.03.010 PMID: 28545793

[59] Elderkin-Thompson, V.; Irwin, M.R.; Hellemann, G.; Kumar, A. Interleukin-6 and memory functions of encoding and recall in healthy and depressed elderly adults. *Am. J. Geriatr. Psychiatry.,* **2012**, *20*(9), 753-763.

[60] Sadhu, A.; Upadhyay, P.; Agrawal, A.; Ilango, K.; Karmakar, D.; Singh, G.P.I.; Dubey, G.P. Management of cognitive determinants in senile dementia of Alzheimer's type: Therapeutic potential of a novel polyherbal drug product. *Clin. Drug Investig.,* **2014**, *34*(12), 857-869. http://dx.doi.org/10.1007/s40261-014-0235-9 PMID: 25316430

[61] Gupta, M.; Kaur, G. Withania somnifera (L.) Dunal ameliorates neurodegeneration and cognitive impairments associated with systemic inflammation. *BMC Complement. Altern. Med.,* **2019**, *19*(1), 217. http://dx.doi.org/10.1186/s12906-019-2635-0 PMID: 31416451

[62] Li, G.; Cherrier, M.M.; Tsuang, D.W.; Petrie, E.C.; Colasurdo, E.A.; Craft, S.; Schellenberg, G.D.; Peskind, E.R.; Raskind, M.A.; Wilkinson, C.W. Salivary cortisol and memory function in human aging. *Neurobiol. Aging,* **2006**, *27*(11), 1705-1714. http://dx.doi.org/10.1016/j.neurobiolaging.2005.09.031 PMID: 16274857

[63] Turpeinen, U.; Hämäläinen, E. Determination of cortisol in serum, saliva and urine. *Best Pract. Res. Clin. Endocrinol. Metab.,* **2013**, *27*(6), 795-801. http://dx.doi.org/10.1016/j.beem.2013.10.008 PMID: 24275191

[64] Blair, J.; Adaway, J.; Keevil, B.; Ross, R. Salivary cortisol and cortisone in the clinical setting. *Curr. Opin. Endocrinol. Diabetes Obes.,* **2017**, *24*(3), 161-168. http://dx.doi.org/10.1097/MED.0000000000000328 PMID: 28375882

[65] Cohen, S.; Kamarck, T.; Mermelstein, R. Perceived stress scale. *Measuring stress: A guide for health and social scientists,* **1994**, *24*(3), 161-168.

[66] Nordin, M.; Nordin, S. Psychometric evaluation and normative data of the Swedish version of the 10-item perceived stress scale. *Scand. J. Psychol.,* **2013**, *54*(6), 502-507. http://dx.doi.org/10.1111/sjop.12071 PMID: 24118069

[67] Nwachukwu, I.; Nkire, N.; Shalaby, R.; Hrabok, M.; Vuong, W.; Gusnowski, A.; Surood, S.; Urichuk, L.; Greenshaw, A.J.; Agyapong, V.I.O. COVID-19 pandemic: Age-related differences in measures of stress, anxiety and depression in Canada. *Int. J. Environ. Res. Public Health,* **2020**, *17*(17), 6366. http://dx.doi.org/10.3390/ijerph17176366 PMID: 32882922

[68] Gopukumar, K.; Thanawala, S.; Somepalli, V.; Rao, T.S.S.; Thamatam, V.B.; Chauhan, S. Efficacy and safety of ashwagandha root extract on cognitive functions in healthy, stressed adults: A randomized, double-blind, placebo-controlled study. *Evid. Based Complement. Alternat. Med.,* **2021**, *2021*, 1-10. http://dx.doi.org/10.1155/2021/8254344 PMID: 34858513

[69] Lin, S.; Hsiao, Y-Y.; Wang, M. *Test Review: The Profile of Mood States,* 2nd ed; SAGE Publications: Los Angeles, CA, **2014**, Vol. 32, pp. 273-277.

[70] Taylor, C.A.; Bouldin, E.D.; McGuire, L.C. Subjective cognitive decline among adults aged ≥45 Years — United States, 2015–2016. *MMWR Morb. Mortal. Wkly. Rep.,* **2018**, *67*(27), 753-757. http://dx.doi.org/10.15585/mmwr.mm6727a1 PMID: 30001562

[71] Brailean, A.; Steptoe, A.; Batty, G.D.; Zaninotto, P.; Llewellyn, D.J. Are subjective memory complaints indicative of objective cognitive decline or depressive symptoms? Findings from the English Longitudinal Study of Ageing. *J. Psychiatr. Res.,* **2019**, *110*, 143-151. http://dx.doi.org/10.1016/j.jpsychires.2018.12.005 PMID: 30639919

[72] Lopresti, A.L.; Smith, S.J.; Ali, S.; Metse, A.P.; Kalns, J.; Drummond, P.D. Effects of a Bacopa monnieri extract (Bacognize®) on stress, fatigue, quality of life and sleep in adults with self-reported poor sleep: A randomised, double-blind, placebo-controlled study. *J. Funct. Foods,* **2021**, *85*, 104671. http://dx.doi.org/10.1016/j.jff.2021.104671

[73] Li, J.; Vitiello, M.V.; Gooneratne, N.S. Sleep in normal aging. *Sleep Med. Clin.,* **2018**, *13*(1), 1-11. http://dx.doi.org/10.1016/j.jsmc.2017.09.001 PMID: 29412976

[74] Cohen, S.; Janicki-Deverts, D. Who's stressed? distributions of psychological stress in the United States in probability samples from 1983, 2006, and 20091. *J. Appl. Soc. Psychol.,* **2012**, *42*(6), 1320-1334. http://dx.doi.org/10.1111/j.1559-1816.2012.00900.x

[75] Cohen, S.; Kamarck, T.; Mermelstein, R. A global measure of perceived stress. *J. Health Soc. Behav.,* **1983**, *24*(4), 385-396. http://dx.doi.org/10.2307/2136404 PMID: 6668417

[76] Adam, E.K.; Kumari, M. Assessing salivary cortisol in large-scale, epidemiological research. *Psychoneuroendocrinology.,* **2009**, *34*(10), 1423-1436. http://dx.doi.org/10.1016/j.psyneuen.2009.06.011 PMID: 19647372

[77] Blessing, E.M.; Reus, V.; Mellon, S.H.; Wolkowitz, O.M.; Flory, J.D.; Bierer, L.; Lindqvist, D.; Dhabhar, F.; Li, M.; Qian, M.; Abu-Amara, D.; Galatzer-Levy, I.; Yehuda, R.; Marmar, C.R. Biological predictors of insulin resistance associated with posttraumatic stress disorder in young military veterans. *Psychoneuroendocrinology,* **2017**, *82*, 91-97. http://dx.doi.org/10.1016/j.psyneuen.2017.04.016 PMID: 28521179

[78] de Assis, G.G.; Gasanov, E.V. BDNF and Cortisol integrative system – Plasticity vs. degeneration: Implications of the Val66Met polymorphism. *Front. Neuroendocrinol.,* **2019**, *55*, 100784. http://dx.doi.org/10.1016/j.yfrne.2019.100784 PMID: 31425696