

Complex Antioxidant Blend Improves Memory in Community-Dwelling Seniors

William K. Summers^{a,*}, Roy L. Martin^a, Michael Cunningham^b, Velda L. DeBoynon^a and Gary M. Marsh^b

^a*Alzheimer's Corporation, Albuquerque, NM, USA*

^b*Graduate School of Public Health, University of Pittsburgh, PA, USA*

Accepted 21 July 2009

Abstract. One hundred thirteen community dwelling subjects between the ages of 50 and 75 without dementia were recruited. A blind administrator randomly assigned 54 subjects to placebo and 59 to active treatment groups. The active treatment consisted of four months treatment with a complex antioxidant blend. Placebo treatment was an identical gel and bottle administered for four months. Forty-eight active subjects and 38 placebo subjects completed the study. Memory testing with a 50 part paired association test and a 20-word immediate recall test were significantly improved, $p = 0.015$ and $p = 0.005$ respectively. A secondary study of serum homocysteine was completed in 25 active treatment subjects and 17 placebo subjects. Significant reduction in serum homocysteine levels was seen in the active treatment subjects ($p = 0.005$). A complex antioxidant blend taken over four months improves performance on two difficult memory tests in community dwelling elderly subjects. Furthermore, the antioxidant significantly reduced the serum homocysteine level in treatment group.

Keywords: Antioxidant, cognitive enhancement, homocysteine, memory, synergistic pharmacology, working memory

INTRODUCTION

Thirty percent of healthy non-demented community-dwelling elderly have complaints of decline in memory function [1]. Cognitive decline from baseline has been seen in 18.2% of older persons followed over three years [2]. Although subjective memory complaints are common in older people and memory complaints predict neuropathologic diseases, they are not always due to neurological disease [1,3,4].

Memory is a nebulous and complex concept. Intelligence and memory are assumed to be related. Yet intelligence is not congruous with memory. Memory is divided into declarative memory, nondeclarative memory, and working memory (immediate recall) [1].

Declarative memory consists of episodic memory and semantic memory. Episodic memory refers to re-

call of personal experiences. Semantic memory refers to “factual information” about the world around us. Going to see the movie “Jaws” is a personal episodic memory. However, recall of the plot of the movie is semantic memory, as the film is available for review to test accuracy. All semantic memory is a product of episodic memory. The inverse is not true.

Declarative memory involves the right superior temporal lobe, the somatosensory parietal cortex, the visual occipital cortex, the left frontal cortex, and both anterior prefrontal regions. Declarative memory requires interplay between these areas and the hippocampus. This is true for both episodic (personally experienced) or semantic (recall of ‘facts’). These structures then process ‘higher cortical functions’. Declarative memory is a more sophisticated function. Declarative memory is tested by paired-word association tests. The test is administered by reading a list of word pairs aloud, and then, after a delay, cueing the subject, with one of the word-pair. For example, a subject might be given the pair, “the COLOR is GREEN”. Later the subject would

*Corresponding author: William K. Summers, M.D., 6000 Up-town Blvd, Suite 308, Albuquerque, NM 87110-4148, USA. Tel.: +1 505 878 0192; Fax: +1 505 888 6000; E-mail: md@abqdhc.com.

be asked “What was the name of the COLOR?” The subject’s correct response will depend on competent interaction between the hippocampus, and several of the other structures given above [2].

Nondeclarative memory refers to capacity to recall processes and actions. Two components of nondeclarative memory are priming memory and procedural memory. Here, seeing a faucet and understanding what it is and how it functions is a priming memory. Choosing to turn on the hot water is a procedural memory. Nondeclarative memory testing has not been standardized to common cognitive deficit conditions, such as Alzheimer’s disease. The Tower of Hanoi task is a well-investigated procedural memory test, but is complex and awkward to administer [3].

Working memory appears to anatomically reside in the hippocampus, amygdala, and associated structures of the medial temporal lobe [2,3]. The competence of working memory can be tested by word list recall, or digit span recall.

Products that improve memory in normal community dwelling elderly are not established. Memory complaints are not a disease state. Products for this symptom rarely fall under the FDA classification of a pharmaceutical. There are numerous health supplements and herbal preparations that claim memory improvement [8,9]. Google search engine produces 304,000 listings for ‘memory supplements’ [10].

The mechanism of action of these memory enhancement products fall into four broad classifications: antioxidants, cerebral blood flow enhancers, neurotransmitter modulators, and lipid supplements. Vitamins and most herbal cognitive enhancers are classified as antioxidants. Cerebral blood flow enhancers include ginseng, gota kola, and vinpocin, which also have antioxidant properties. Neuropeptides, the herb Huperzine A (fir moss or fir clubmoss), and nicotine would be typical neurotransmitter modulators. Examples of lipid supplements are choline, phosphatidylserine, and omega 3 lipids. Many lipid compounds also have antioxidant properties.

Antioxidants have five forms [11,12]. First are antioxidant vitamins. Examples are beta carotene, vitamin A, Coenzyme Q10, vitamin B2, folic acid, vitamin B3, vitamin B5, vitamin B6, vitamin B12, vitamin C, and vitamin E. The second are antioxidant amino acids, like L-glutathione, L-lysine, L-methionine, and taurine. Third are antioxidant minerals, such as boron, manganese, magnesium, selenium, and zinc. Fourth are antioxidant herbals. There are numerous examples, such as curcumin, ginkgo biloba, ginseng, gota

kola, and blueberry (*vaccinium angustifolium*). Fifth are the antioxidant lipids, such as lipoic acid, phosphatidylcholine, phosphatidylserine, and phosphatidylethanolamine.

Basic animal research concludes that antioxidants, in adequate dosage, improves cognitive performance [13, 14]. Blueberry extract, for example, not only improves memory tasking, but it also inhibits acetylcholinesterase, a synaptic enzyme which is inhibited by therapies for Alzheimer’s disease, such as tacrine, donepezil, and rivastigmine [13,14]. Further, it would appear that blueberry extracts decrease oxidative DNA damage, a marker of aging, in the liver [15]. Ginkgo biloba extract enhances hippocampal neurogenesis in mice [16].

In humans, the literature on use of antioxidants has been generally negative [14,17–19]. More careful examination of the literature reveals that the focus has been on mono-vitamin or duo-vitamin therapy with vitamin E and/or vitamin C [14,18,19]. This approach is naive and flawed. For example, the studies of vitamin E do not address all forms of that vitamin. In plants, there are eight forms of vitamin E. Commonly, alpha-tocopherol is used in research as a single agent, but alpha-tocopherol is 60% less potent than alpha-tocotrienol [20]. To date most human successful studies have used herbals, fruits, and vegetables [21–24], however, eating the large quantities of fruits, herbs, and vegetables required to get a pharmacologic effect is neither practical nor cost effective.

The purpose of this study is to evaluate the effect on memory capacity of a potent complex antioxidant on non-demented community dwelling seniors over a four-month period. The complex antioxidant blend used contained 34 antioxidants representing all five classes of antioxidants [11]. This blend of antioxidants includes phospholipids, the building block of cell membranes.

MATERIALS AND METHODS

Subjects

One hundred thirteen subjects were recruited from the general Albuquerque metropolitan area. Minimum exclusion criteria were applied. Subjects were to be non-demented and living independently in the community and between the ages of fifty and seventy-five. Memory testing was in English. Subjects had to be English speaking. Exclusion criteria included living in a structured community, such as a nursing home or

assisted living. Subjects could not be in a hospice program. Subjects could not be taking coumadin, anti-cancer drugs, antipsychotics, corticosteroids, or anti-dementia drugs. Subjects could not be on a continuous positive airway pressure (CPAP) device or have known obstructive sleep apnea syndrome. Subjects were not paid to participate, nor reimbursed for expenses.

Procedure

This experimental protocol complied with guidelines on human experimentation. Independent Review Consulting Inc. (Corte Madera, CA) approved Informed Consent procedures and forms. Financial support for this research was obtained from Solo Non-Profit Research, Ltd., Arcadia, CA.

After explanation of the protocol to a subject, informed consent was obtained. The intake interview recorded demographic data, medical history, and complete listing of prescribed medications, over-the-counter medications, and health supplements. Baseline age, height, weight, waist size, gender, and race data were collected. Race was self-reported by the subject. A baseline battery of memory tests was administered. An administrator, who never met or tested subjects, randomly assigned the subject by a computer generated program to either placebo or active treatment group.

In the baseline interview, health supplements were reviewed in detail. Labels of supplement bottles were analyzed, when available. If daily supplement ingestion appeared to exceed the following doses in more than two supplements, the subject was considered a sophisticated health supplement subject (SHS). These parameters were vitamin B2 (riboflavin) > 1.7 mg per day, vitamin B6 (pyridoxine) > 3 mg per day, vitamin B9 (folic acid) > 400 mcg per day, or vitamin B12 (methylcobalamin) > 18 mcg per day. The parameters were arbitrarily selected from a typical over the counter multivitamin.

Subjects were interviewed one month after intake interview. The focus was on any compliance or complication issues. Subjects were asked to bring in the study drug bottles for inspection. Any significant changes in health status or medications were noted. The memory test battery was repeated.

The final interview was conducted four months after the initial interview. The medicine and health information was updated. The memory battery was administered. When appropriate, the second serum sample was collected to be tested for homocysteine.

Memory testing

The memory test battery included the Mini-Mental Status Examination (MMSE) and an expanded 50-item Names-Learning paired association test (NLT₅₀) [25, 26]. The latter instrument was designed to have little ceiling effect and to detect subtle memory deficits. Normal is 81 out of 100 (SD = 4.2). Finally, the 20-word free-recall test (₂₀WRT) of short-term memory was given [27]. Subjects were presented with a list of 20 one or two syllable unrelated words, at two second intervals. They were then asked to recall words in any order within two minutes. The maximum possible score is 20 with a mean of 6.86 words (SD = 2.45). The entire battery takes about 30 minutes to administer.

Treatments

The administrator mailed the active treatment group subjects bottles of the 34-component blend of antioxidants [28]. Table 1 lists the active treatment formulation [24,27]. Treatment consisted of ingestion of six No. 20 gels of the active treatment per day.

The placebo group subjects were mailed bottles of identically packaged and appearing No. 20 size gels and instructed to ingest six gels per day.

Homocysteine sub-study

A sub-study of serum homocysteine effects of treatment was conducted in the first 50 subjects to volunteer for a blood draw at baseline and at four months. Limited finances allowed only the first fifty volunteer subjects to participate in this sub-study.

Statistical analysis

Statistical analyses were conducted to test for differences in the baseline characteristics of the members in the active and placebo groups. The two sample t-test was used for continuous variables and Pearson's chi-squared test was used for categorical variables.

Change scores were calculated for the difference in the names learning test and 20 word recall scores at four months compared to baseline. To test the null hypothesis of equality in the change scores in the active and placebo groups versus the alternative of unequal change scores, the two sample t-test was used. For the names-learning test, the score variances were significantly different. Therefore, Satterthwaite's approxima-

Table 1
Composition of 34 component antioxidant blend

Component	Daily dose	% Daily value	Classification
Alpha lipoic acid	90 mg		Lipid antioxidant
d-alpha tocopherol	240 IU	2,400%	Vitamin antioxidant
Ascorbic acid	300 mg	500%	Vitamin antioxidant
A vitamin palmitate	4,500 IU	450%	Vitamin antioxidant
Beta carotene	9,000 IU	900%	Vitamin antioxidant
Bioflavonoid (lemon)	90 mg		Herbal antioxidant
Boron citrate	60 μ g		Mineral antioxidant
Co-enzyme Q10	36 mg		Lipid antioxidant
Copper gluconate	75 μ g	180%	Mineral antioxidant
DMAE	67.5 mg		Lipid antioxidant
Eleutherococcus senticosus	90 mg		Herbal antioxidant
Folic acid	720 μ g	180%	Vitamin antioxidant
Gingko biloba	90 mg		Herbal antioxidant
Ginseng (10% ginsenosides)	90 mg		Herbal antioxidant
<i>l</i> -glutathione	120 mg		Amino acid antioxidant
Gotu kola	120 mg		Herbal antioxidant
Grape seed extract	210 mg		Herbal antioxidant
<i>l</i> -lysine	180 mg		Amino acid antioxidant
Magnesium citrate	48 mg	14%	Mineral antioxidant
Manganese citrate	3 mg	67%	Mineral antioxidant
<i>l</i> -methionine	180 mg		Amino acid antioxidant
Methylcobalamin	720 μ g	3,000%	Vitamin antioxidant
Nicotinamide	24 mg	150%	Vitamin antioxidant
Pantothenate, <i>d</i> -calcium	60 mg	1,200%	Vitamin antioxidant
Papain	9 mg		Herbal antioxidant
Phosphatidylcholine	480 mg		Lipid antioxidant
Phosphatidylserine	30 mg		Lipid antioxidant
Pyridoxine HCl	18 mg	1,059%	Vitamin antioxidant
Pyridoxal-5- phosphate	3.6 mg	212%	Vitamin antioxidant
Riboflavin-5- phosphate	6 mg	462%	Vitamin antioxidant
<i>l</i> -selenomethionine	60 μ g	100%	Mineral antioxidant
Taurine	90 mg		Amino acid antioxidant
Thiamine	24 mg	2,000%	Vitamin antioxidant
Tocotrienols, mixed	186 mg		Vitamin antioxidant
Zinc citrate	18 mg	120%	Mineral antioxidant

tion was used. For all comparisons, *p*-values of less than 0.05 were considered statistically significant.

Univariate linear regression models were run to individually test whether or not treatment, gender, race, age, or other member characteristics were significant predictors of change score for the 20 word recall and names learning test. To adjust for other potential confounding variables in the comparison of the change scores, multi-variable linear regression models were calculated. Forward stepwise linear regression (with *p*-value for entry of 0.20) was used to determine which covariates should be included in the multi-variable models. For the final models of the change score outcomes, the treatment effects were adjusted for gender, race, and age.

RESULTS

One hundred and thirteen subjects enrolled in the study. Eleven (9.7%) were referred from the principle investigator's medical practice. Eighty-two subjects (72.6%) joined the study as a result of advertising. Twenty subjects (17.7%) were referred to the study by word of mouth.

Fifty-four were assigned to the placebo group and 59 were in the active treatment group. There were no significant differences between the treatment and placebo groups for demographic and other parameters. These included age, height, weight, waist size, gender, race, level of education, or whether the subjects were on prescriptions. Completion of the study (4 months)

Table 2
Characteristic of Subjects completing four months of study

Characteristic	Placebo (<i>n</i> = 38)	Active (<i>n</i> = 48)	p-value ^a
Age, mean (SD)	60.0 (7.0)	63.3 (6.9)	0.031
Height, mean (SD)	65.4 (5.9)	65.5 (5.8)	0.958
Weight, mean (SD)	166.5 (29.3)	169.3 (36.8)	0.704
Waist, mean (SD)	37.0 (4.4)	37.5 ^b (4.7)	0.658
Gender, n (%)			
Female	24 (63.2)	26 (54.2)	0.401
Male	14 (36.8)	22 (45.8)	
Race, n (%)			
White	30 (79.0)	37 (77.1)	0.836
Nonwhite	8 (21.0)	11 (22.9)	
How participant heard of trial, n (%)			
Ads	27 (71.1)	32 (66.7)	0.876
Clinic	4 (10.5)	5 (10.4)	
Word of Mouth	7 (18.4)	11 (22.9)	
Prescription, n (%)			
No	38 (100.0)	48 (100.0)	1.000
Yes	0 (0.0)	0 (0.0)	

^a. Comparison of placebo to active group (null hypothesis of equality between groups).

Two-sample t test for continuous variables and chi-square test for categorical variables.

^b. *n* = 47.

was achieved by 38 of the 54 placebo subjects (70.4%). Eight withdrew from the study, and eight were removed for non-compliance to protocol. Of the 59 active treatment group, 48 (81.4%) of the subjects completed the study. Six withdrew from the study and eight were removed for non-compliance to protocol. Analysis of the characteristics of individuals who completed the four months is given in Table 2. Although there were no significant differences between groups at baseline, one demographic, age, differed between the active and placebo groups in those who completed the study ($p = 0.031$). As this difference was not present on enrollment, no correction was made. Further, the older age of the treatment group (averaging 3.3 years older) would bias *against* a positive treatment effect by the health supplement. Adjusted regression analysis controlled for age concluded that this difference did not significantly influence the primary outcomes.

Of subjects completing the four-month protocol, baseline MMSE mean score was 29.1 of a 30 possible score for placebo subjects and 29.3 for active subjects. No subject scored below 27. No subject met criteria for dementia or mild cognitive impairment [26,29]. Repeat MMSE conducted at four-month follow-up. Placebo group average MMSE score was 29.3. Active treatment group MMSE score at four months was 29.7. At four-month examination, no subject scored below 27. No subject met criteria for dementia.

Figure 1 displays mean NLT₅₀ scores at baseline and at four months. The placebo group moved from baseline 69.3 (SD = 14.7) to a four-month mean NLT₅₀

of 72.3 (SD = 13.2). The active treatment group had a NLT₅₀ change from 72.3 (SD = 13.0) at baseline to 79.0 (SD = 15.4) after four months. The mean change in NLT₅₀ scores from four months to baseline in the active versus placebo groups was 3.7 (95% CI = -0.23, 7.65, two-sided $p = 0.065$). The adjusted linear regression modeling of the change in NLT₅₀ scores from four months to baseline revealed a statistically significant 5.2 point higher change in the active group compared to the placebo group ($p = 0.015$).

Figure 2 displays the mean ₂₀WRT scores at baseline and at four months. The placebo group moved from a baseline of 7.0 (SD = 2.3) to 8.2 (SD = 2.6) words recalled from the list of 20 after four months. The active treatment group improved from 7.0 (SD = 2.3) at baseline to a mean of 9.7 (SD = 2.7) ₂₀WRT at the four month interview. The mean change in ₂₀WRT scores from four months to baseline in the active versus placebo groups was 1.4 (95% CI = 0.26, 2.63, two-sided $p = 0.017$). The adjusted linear regression modeling of the change in ₂₀WRT from 4 months to baseline revealed a statistically significant 1.7 point higher change in the active group compared to the placebo group ($p = 0.005$).

Fifty-three subjects volunteered for the analysis of serum homocysteine levels. Of these 29 were on active treatment, while 24 subjects were on placebo treatment. There were no significant differences between the two groups by gender, age, race, height, weight, or waist measurement, as demonstrated in Table 3. Review of the medication and supplements of these 53 subjects

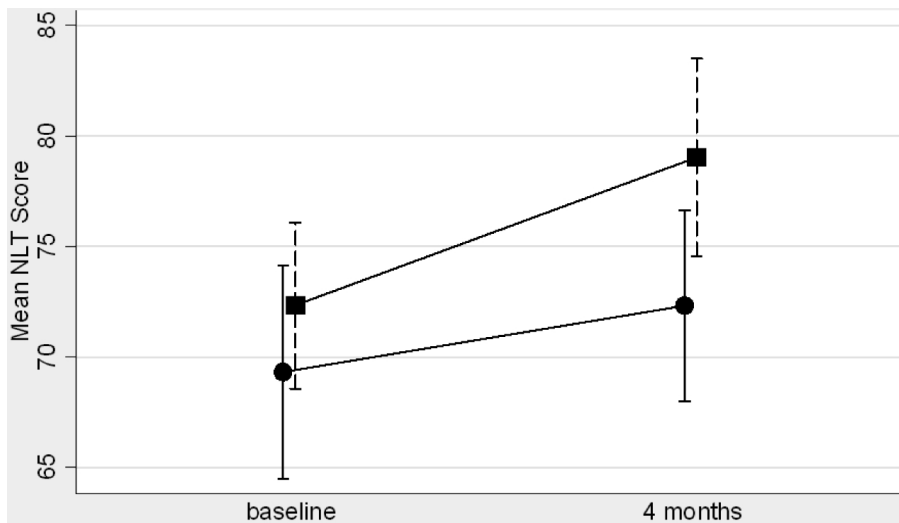


Fig. 1. Unadjusted Mean NLT₅₀ Scores at baseline and four months for placebo (●) and active treatment (■). 95% confidence levels are given.

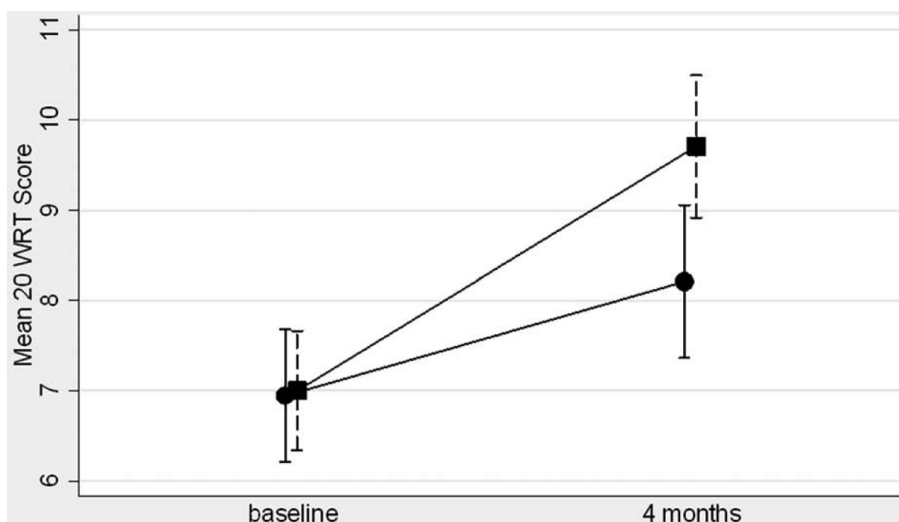


Fig. 2. Unadjusted Mean 20 WRT scores at baseline and 4 months for placebo (●) and active treatment (■). 95% confidence levels are given.

showed no significant difference in the number who were on more sophisticated health supplements (SHS subjects). Serum homocysteine levels were drawn at baseline and at four-month follow-up. Of the 29 active treatment subjects, 25 had the four-month homocysteine level drawn. Of the 24 placebo subjects who had baseline homocysteine levels taken, 17 subjects completed the study.

Figure 3 displays the change in mean homocysteine levels for the sub-study active treatment and placebo treatment groups. The completing active treatment

group was slightly older than placebo subjects. This was not significant. Active treatment subjects also had a slightly higher baseline homocysteine level. This was also not significant. The two-sample t-test for the mean change in homocysteine in the active versus the placebo group did reveal a statistically significant 1.57 $\mu\text{mol/liter}$ decrease after 4 months (95% CL = $-2.72, -0.42$, two-sided $p = 0.009$).

Further analysis revealed six of the 17 placebo subjects completing the homocysteine analysis were SHS subjects. Eight of 25 active treatment subjects were

Table 3
Characteristic of subjects completing 4 months of homocysteine study

Characteristic	Active (n = 25)	Placebo (n = 17)	p-value ^a
Age, mean (SD)	63.9 (5.7)	60.7 (7.2)	0.114
Height, mean (SD)	65.0 (6.8)	63.9 (7.6)	0.630
Weight, mean (SD)	178.6 (36.9)	162.1 (28.3)	0.129
Waist, mean (SD)	38.5 (5.0)	36.1 (4.2)	0.268
Gender, n (%)			
Female	14 (56.0)	11 (64.7)	0.573
Male	11 (44.0)	6 (35.3)	
Race, n (%)			
White	20 (80.0)	15 (88.2)	0.482
Other	5 (8.0)	2 (11.8)	
How subject heard of trial, n (%)			
Ads	17 (68.0)	14 (82.4)	0.579
Clinic	3 (12.0)	1 (5.9)	
Word of mouth	5 (20.0)	2 (11.8)	
Prescription, n (%)			
No	25 (100.0)	17 (100.0)	1.000
Yes	0 (0.0)	0 (4.2)	
Vitamin taker, n (%)			
No	17 (68.0)	11 (64.7)	0.824
Yes	8 (32.0)	6 (35.3)	

a. Comparison of placebo to active group (null hypothesis of equality between groups). Two-sample t test for continuous variables and chi-square test for categorical variables.

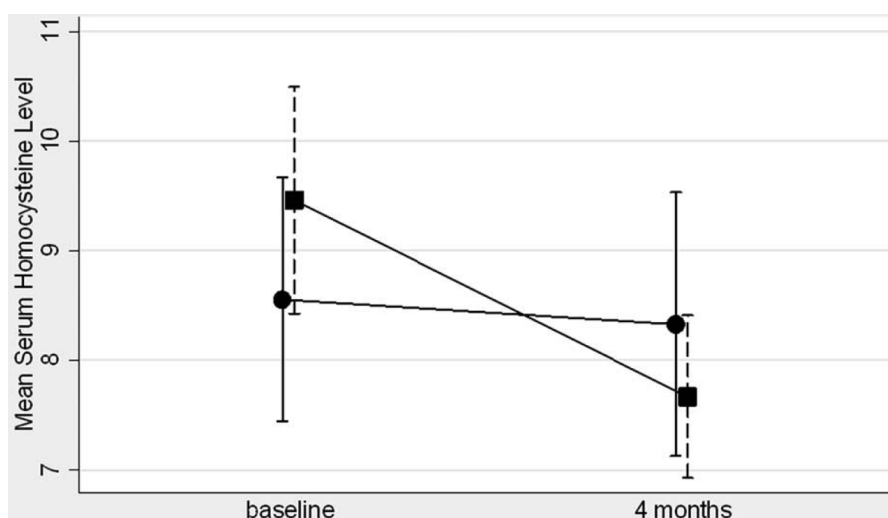


Fig. 3. Sub-study of serum homocysteine changes: Unadjusted mean serum homocysteine levels in micromolar/liter at baseline and four months for placebo (●) and active treatment (■). 95% confidence levels are given.

SHS subjects. Despite the small number of observations in these sub-groups it appeared that the active treatment resulted in substantial lowering of serum homocysteine. Among non-supplement takers, active treatment resulted in a 1.10 $\mu\text{mol/liter}$ mean decrease in homocysteine (two sided $p = 0.084$).

Among vitamin takers, the mean change at four months in the active group was 2.56 $\mu\text{mol/liter}$ lower than the mean change in the placebo group (two-sided $p = 0.046$).

DISCUSSION

Cognitive functioning is believed to decline across life span and is generally referred to as age-related cognitive decline [5]. Mild cognitive impairment in the elderly is believed to progress to Alzheimer's disease [4, 5, 7, 25, 30]. Agents that could reverse or slow cognitive decline would be of potential value to the aging population.

This study evaluated the effect of a 34 component complex antioxidant on memory of non-demented community dwelling subjects between the age of 50 and 75. The results show significant improvement after four months of treatment for both memory tasks. The increase in mean change in NLT₅₀ test score in the active group was significant at a p-value = 0.015. The NLT₅₀ is believed to test declarative memory, which is a higher cortical function. Declarative memory involves the right superior temporal lobe, the somatosensory parietal cortex, and visual occipital cortex, left frontal cortex, and both anterior prefrontal regions. The increase in the ₂₀WRT favored the active complex antioxidant treatment ($p = 0.005$). The ₂₀WRT putatively reflects working memory or hippocampal function.

This study has strengths and limitations. The strengths are a study population with few exclusions and simple memory testing instruments. The period of study, four months, is appropriate to look for memory improvements from a health supplement. Use of only two testing personnel with use of both testing staff for each subject added to the consistency of the data.

A weakness of the study was the absence of memory testing at two and three months. The specificity of the memory tests is assumed from fMRI research. However, research in this field is not settled science.

Memory and antioxidants

The current literature would suggest that these results are surprising. Kamat and colleagues [14] recently reviewed twenty years of antioxidant use in human and animal studies. They concluded that the theory of antioxidant causation of neurological conditions was sound, but that the implementation of antioxidant therapies in humans 'must be flawed.' We concur.

First, prior studies of supplements employ massive amounts of single or simple combinations of antioxidants. This 'pharmacologic approach' would assume that aging affects only a small number of metabolic sites. But, biological milieus are complex and diverse.

High dose, short-term simple supplement treatments are unlikely to result in benefit. The avoidance of complex combinations of active agents is understandable. The literature is biased against "poly-pharmacy". Yet "poly-pharmacy" can result in neutral effect, synergistic effect, or antagonist effect. Classical pharmacology teaches that synergistic combinations allow use of smaller doses of each component. Combining synergistic antioxidants agents that act at multiple metabolic levels is more likely give benefit.

In the present study, the active treatment was a complex antioxidant blend (Table 1). The formulation was designed to impart synergistic benefit. Anticipated was lower effective doses of individual components with less potential side effect.

Improving cerebral vascular flow was intended by inclusion of *eleutherococcus senticosus*, *ginkgo biloba*, *panax quiquefolium*, *centella asiatica*, grape pip (*vide supra*) and magnesium citrate. Prevention and repair of lipid peroxidation was addressed by inclusion of alpha-lipoic acid, d-alpha tocopheryl, mixed tocopherols, ascorbic acid, vitamin A palmitate, beta-carotene, lemon bioflavonoids, L-glutathione, l-lysine, phosphatidylcholine, phosphatidylserine, pyridoxine, riboflavin-5-phosphate, and selenium methiodine. Enhancement of superoxide dismutase and reduction of serum homocysteine was targeted with copper sebacate, folic acid, manganese citrate, l-methionine, methylcobalamin, and pyridoxine. Addressing free radicals of nuclear origin, mitochondrial metabolism, or post translational protein production was intended by inclusion of ascorbic acid, beta carotene, boron, coenzyme Q10 folic acid, magnesium citrate, methylcobalamin, nicotinamide, pyridoxal 5'-phosphate, and taurine. Other positive synergies exist within the formulation. It is beyond the scope of this paper to describe or fully reference molecular and metabolic mechanisms of all components of the formulation [12,31,32].

A second reason that prior antioxidant studies in humans have failed to show memory improvement is the crude instruments employed. Most popular cognitive testing instruments were developed before it was suspected that memory problems could be reversed. The original intent of the test was to diagnose a condition, not to measure improvement. For example, the Longitudinal Aging Study Amsterdam used the MMSE to measure cognitive decline [5]. Yet the MMSE suffers from both floor and ceiling effects, making it a crude unreliable tool [25]. Some studies use a numbing battery of "cognitive tests" to measure change [30]. These ordeals often take hours, which taint the data by testing the subject's response to fatigue. For example, the Boston Naming Test was developed for assessment of aphasia, has a 135-page instruction book, and requires 45 minutes to complete [33].

In the present study, the three tests were administered in less than 30 minutes. The MMSE was considered a diagnostic test. The MMSE was not developed as a valid tool to measure change or improvement. The NLT₅₀ addresses higher cognitive centers linking left frontal lobes and occipital lobe to hippocampus and

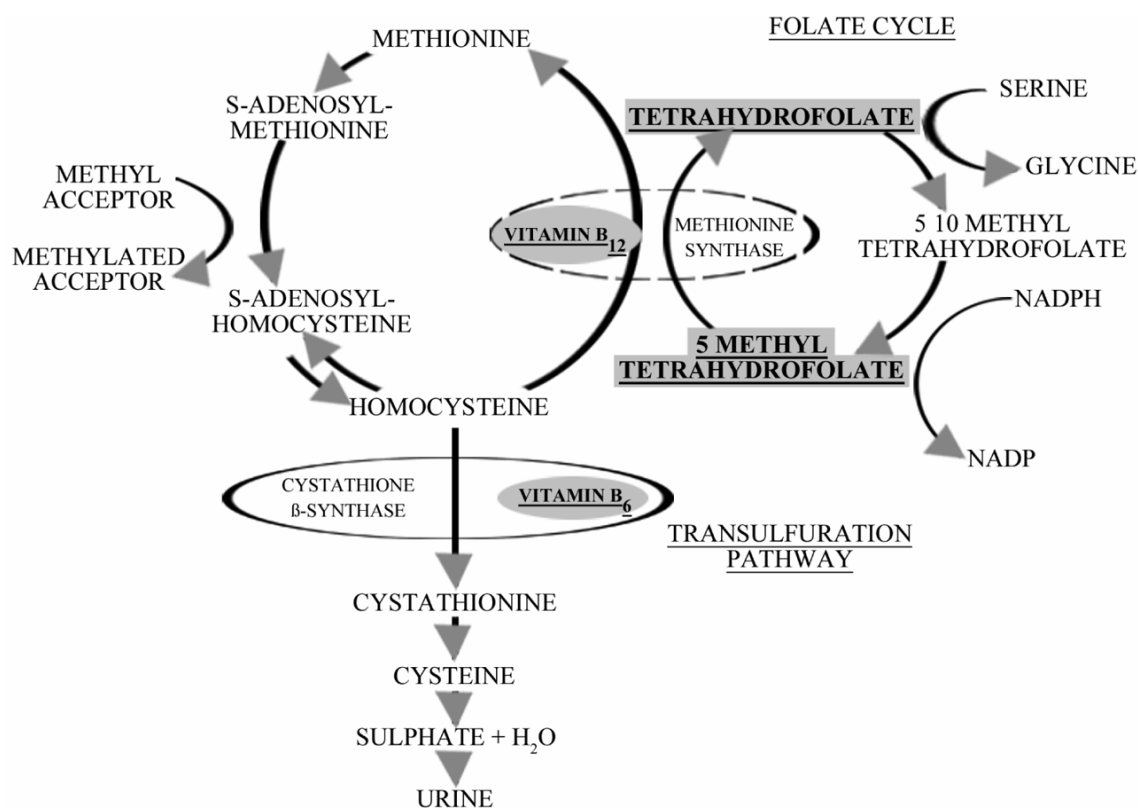


Fig. 4. Homocysteine metabolism. Note the key role of B12 in the folate cycle regenerating homocysteine to methionine, and the role of B6 in converting homocysteine to cystathionine.

superior temporal lobe [2,34]. Because there were four versions of ${}_{20}$ WRT and NLT ${}_{50}$, long term memory was minimally involved.

A third cause of negative human results when testing antioxidant effect on memory is the 'Epic Research Project' [16,35–37]. Here, multiple researchers spread over vast distances administer remarkably complex protocols. Consistency of such protocols is problematic. The duration of the studies frequently are over three years, making subject compliance a serious issue. In all, such epic protocols will drift toward non-significance.

In the current study, the population is manageably small and the protocol compliance is minimally challenging. True, the study funding and staff are linked to the company that makes the study drug, but these biases are openly displayed.

In the field of Alzheimer's disease, there are recent well-designed small studies using combinations of vitamins and nutraceuticals. In one study, community-dwelling subjects with early stage Alzheimer's disease improved in a one-year study [38]. In another

study, vitamins with nutraceuticals were used to treat moderate and late stage Alzheimer's disease over nine months [39]. A third study used the herbal, panax ginseng, in a double blind study of 97 subjects to successfully treat Alzheimer's patients [40].

Homocysteine

Homocysteine has been linked to mild cognitive impairment and other aspects of cognitive decline [36, 41]. Initially, high plasma total homocysteine was linked to atherothrombotic disease [42]. B complex vitamins (pyridoxine, folic acid and cyanocobalamin) lower homocysteine levels [43]. Metabolically vitamin B6 (pyridoxal phosphate) is a key cofactor in the transfer of sulphhydryl groups from methionine to serine via methionine synthase conversion of 5-methyl-tetrahydrofolate (Fig. 4). Vitamin B6 also transfers sulphhydryl groups from homocysteine to cystathionine, allowing elimination of sulphate (S04) via the urine. The role of vitamin B6 is principal in the reduction of homocysteine. However, Fig. 4 also demonstrates the

importance of vitamin B9 (tetrahydrofolate, 5-methyl THF, and 10 formyltetrahydrofolate) and vitamin B12 (methyl cobalamin and adenosyl cobalamin).

In the present study, the study drug contains supplements that should reduce serum homocysteine. Comparison of 29 active treatment subject volunteers to 24 placebo subject volunteers showed significant reduction of serum homocysteine levels ($p = 0.005$). This reduction held even if the subject was already on B complex vitamins beyond those offered in common multivitamins.

A recent multi-center study concluded that reducing homocysteine levels with high-dose B vitamins does not slow cognitive decline in mild to moderate Alzheimer's disease individuals [36]. However, this study had serious drawbacks: 1) the study populations were already suffering from Alzheimer's disease; 2) the baseline homocysteine levels were normal; 3) the dose of vitamins was unbalanced; 4) the duration of follow-up was short; and 5) measurement tools were problematic. Earlier studies, linking Alzheimer's disease and cognitive function in non-demented individuals to homocysteine levels, have better designs [44, 45].

In the present study, the antioxidant blend proved most potent in reducing the serum homocysteine, even in those subjects already taking vitamins typically associated with reduced homocysteine level. Perhaps inclusion of copper sebacate, manganese citrate, l-methionine, and zinc might explain this finding. However, this side study may suffer from small sample size. It remains to be determined if lower serum homocysteine levels are related to the memory improvement seen in the study or if it might be related to long-term health.

CONCLUSION

In summary, use of a component antioxidant blend demonstrated improved memory in a double blind study of 113 generally healthy community dwelling individuals who did not have dementia. In a sub-study of 53 subjects, the active agent significantly reduced the serum homocysteine level.

DISCLOSURE STATEMENT

Authors' disclosures available online (<http://www.jalz.com/disclosures/view.php?id=113>).

REFERENCES

- [1] Cargin JW, Maruff P, Collie A, Shafiq-Antonacci R, Masters C (2007) Decline in verbal memory in non-demented older adults. *J Clin Exp Neuropsychol* **29**, 706-718.
- [2] Comijs HC, Dik MG, Deeg DJ, Jonker C (2004) The course of cognitive decline in older persons: results from the longitudinal aging study Amsterdam. *Dement Geriatr Cogn Disord* **17**, 136-142.
- [3] Vogel A (2008) Subjective memory complaints in older people. Is it a symptom of dementia? *Ugeskr Laeger* **170**, 1728-1733.
- [4] Jorm AF, Masaki KH, Davis DG, Hardman J, Nelson J, Markesbery WR, Petrovitch H, Ross GW, White LR (2004) Memory complaints in non-demented men predict future pathologic diagnosis of Alzheimers disease. *Neurology* **63**, 1960-1961.
- [5] Stern Y, Sackeim HA (2008) Neuropsychiatric aspects of memory and amnesia. In *Neuropsychiatric and Behavioral Neurosciences (5th edition)*, Yudofsky SC, Hales RE, eds. American Psychiatric Publishing Inc., Washington, DC, pp. 567-575.
- [6] Mitchell TM, Shinkareva SV, Carlson A, Chang K, Malave VL, Mason RA (2008) Predicting human brain activity associated with meanings of nouns. *Science* **320**, 1191-1195.
- [7] Cohen NJ (1984) Preserved learning capacity in amnesia: evidence for multiple memory systems. In *The Neuropsychology of Memory*, Squire LR, Buters N, eds. Guilford Press, New York, pp. 83-103.
- [8] Smith KL (2007) *Brighter Mind*. Brighter Mind Media Group, Corinth TX, pp. 203-292.
- [9] Dean W, Morgenthaler J, Fowkes SW (1993) *Smart Drugs II*. Smart publications, Petaluma, CA, pp. 1-274.
- [10] Google search for "memory supplement", <http://www.google.com/search?hl=en&q=memory+supplement>, Accessed on April 13, 2009.
- [11] Summers WK (2004) Alzheimer's disease, oxidative injury, and cytokines. *J Alzheimers Dis* **6**, 651-657.
- [12] Halliwell B, Gutteridge JMC (2001) *Free Radicals in Biology and Medicine (3rd Edition)*. Oxford University Press, Oxford pp.105-245.
- [13] Papandreou MA, Dimakopoulou A, Linardaki ZI, Cordopatis P, Klimis-Sacas D, Margariti M, Laman FN (2009) Effect of polyphenol-rich wild blueberry extract on cognitive performance of mice, brain antioxidant markers and acetylcholinesterase activity. *Behav Brain Res* **198**, 352-358.
- [14] Kamat CD, Gadal S, Mhatre M, Williamson KS, Pye QN, Hensley K (2008) Antioxidants in central nervous system diseases: Preclinical promise and translational challenges. *J Alzheimers Dis* **15**, 473-493.
- [15] Dulebohn RV, Yi W, Srivastava A, Akoh CC, Krewer G, Fischer JG (24 DEC 2008) Effects of blueberry (*vaccinium ashei*) on DNA damage, lipid peroxidation and phase II enzyme activities in rats. *J Agric Food Chem* **56**, 11700-117006.
- [16] Tchanchou F, Xu Y, Wu Y, Christen Y, and Luo Y (2007) EGb761 enhanced adult hippocampal neurogenesis and phosphorylation of CREB in transgenic mouse model of Alzheimer's disease. *FASEB J* **21**, 2400-2408.
- [17] DeKosky ST, Williamson JD, Fitzpatrick AL, Kronmal RA, Ives DG, Saxon JA, Lopez OL, Burke G, Carlson MC, Fried LP, Kuller LH, Robbins JA, Tracy RP, Woolard NF, Dunn L, Snitz BE, Nahin RL, Furberg CD (2008) *Ginkgo biloba* for prevention of dementia: a randomized controlled trial. *JAMA* **300**, 2253-2262.

- [18] Petersen RC, Thomas RG, Grundman M, Bennett D, Doody R, Ferris S, Galasko D, Jin S, Kaye J, Levy A, Pfiffer E, Santo M, vanDyke CH, Thal LJ (2005) Vitamin E and donepezil for the treatment of mild cognitive impairment. *N Engl J Med* **352**, 2379-2388.
- [19] Luchsinger JA, Tang MX, Shea S, Mayeux R (2003) Antioxidant vitamin intake and risk of Alzheimer disease. *Arch Neurol* **60**, 203-208.
- [20] Tan B (2005) Appropriate spectrum vitamin E and new perspective on desmethyl-tocopherols and tocotrienols. *J Nutraceuticals Nutr* **8**, 35-42.
- [21] Rai SS, Shovlin C, Wesnes KA (1991) A double-blind placebo controlled study of *Ginkgo biloba* extract (tanakan) in elderly outpatients with mild to moderate memory impairment. *Curr Med Res Opin* **12**, 350-355.
- [22] Joseph JA, Shukitt-Hale B, Denisova NA, Bielinski D, Martin A, McEwen JJ, Bickford PC (1999) Reversals of age-related declines in neuronal signal transduction cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. *J Neurosci* **19**, 8114-8121.
- [23] Schnyder G, Roffi M, Flammer Y, Pin R, Hess OM (2002) Effect of homocysteine lowering therapy with folic acid, vitamin B12, and vitamin B6 on clinical outcome after percutaneous coronary intervention: the Swiss heart study. *JAMA* **288**, 973-979.
- [24] Fairfield KM, Fletcher RH (2002) Vitamins for chronic disease prevention in adults. *JAMA* **287**, 3116-3126.
- [25] Summers WK, DeBoynton VL, Marsh GM, Majovski LJ (1990) Comparison of seven psychometric instruments used for evaluation of treatment effect in Alzheimer's disease. *Neuroepidemiology* **9**, 193-207.
- [26] Folstein MF, Folstein SE, McHugh PR (1975) Mini-mental state: a practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* **12**, 189-198.
- [27] Singh-Manoux A, Richards M, Marmot M (2003) Leisure activities and cognitive function in middle age: evidence from the Whitehall II study. *J Epidemiology Community Health* **57**, 907-913.
- [28] Memory reVITALIZER®. [http://www. MemoryreVITALIZER.com](http://www.MemoryreVITALIZER.com).
- [29] American Psychiatric Association (1994). *Diagnostic and Statistical Manual of Mental disorders* (4th ed). American Psychiatric Publishing Inc., Washington, DC.
- [30] Petersen RC (2004) Mild cognitive impairment as a diagnostic entity. *J Int Med* **256**, 183-194.
- [31] Packer L, Nam Ong C, Halliwell B, eds (2004) *Herbal and Traditional Medicine*. Marcel Dekker, New York., pp.1-86; 145-164; 489-536.
- [32] Gruenwald J, Brendler T, Jaenicke C, eds (1998) *Physicians Desk Reference for Herbal Medicines*. Medical Economics Co, Montvale, NJ.
- [33] Boston Diagnostic Aphasia Examination, 3rd ed. <http://www3.parinc.com/products/product.aspx?productid=BDAE>. Accessed April 13, 2009.
- [34] Gluck M, Mercado E, Myers CE (2008) Chapter 3: Episodic and semantic memory In *Learning and Memory 1st edition*. Worth Publishers, New York, pp.83-122.
- [35] Grodstein F, Chen J, Willett WC (2003) High-dose antioxidant supplements and cognitive function in community dwelling elderly women. *Am J Clin Nutr* **77**, 975-84.
- [36] Aisen PS, Schneider LS, Sano M, Diaz-Arrastia R, van Dyck CH, Weiner MF, Bottiglier T, Jin S, Stokes KT, Thomas RG, Thal LJ (2008) High-dose B vitamin supplementation and cognitive decline in Alzheimer disease: a randomized controlled trial. *JAMA* **15**, 1774-1783.
- [37] Jamison RL, Hartigan P, Kaufman JS, Goldfarb DS, Warren SR, Guarino PD, Gaziano JM (2007) Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease: a randomized controlled trial. *JAMA* **298**, 1163-1170.
- [38] Chan A, Paskavitz J, Remington R, Rasmussen S, Shea TB. (2008) Efficacy of vitamin/nutriceuticals formulation for early-stage Alzheimer's disease: a 1-year, open-label pilot study with an 16-month caregiver extension. *Am J Alzheimers Dis Other Demen* **23**, 571-585.
- [39] Remington R, Chan A, Paskavitz J, Shea TB (2009) Efficacy of a vitamin/nutriceuticals formulation for moderate-stage to later-stage Alzheimer's disease: a placebo-controlled pilot study. *Am J Alzheimers Dis Other Demen* **23**, 571-585.
- [40] Lee ST, Chu K, Sim JY, Heo JH, Kim M. (2008) Panax ginseng enhances cognitive performance in Alzheimer disease. *Alzheimer Dis Assoc Disord* **22**, 222-226.
- [41] Schafer JH, Glass TA, Bolla KI, Mintz M, Jedlicka AE, Schwartz BS (2005) Homocysteine and cognitive function in a population-based study of older adults. *JAGS* **53**, 381-388.
- [42] McCully KS (1969) Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. *Am J Pathol* **56**, 111-128.
- [43] Wilcken DE, Wilcken B (1997) Natural history of vascular disease in homocysteinuria and the effects of treatment. *J Inherit Metab Dis* **20**, 295-300.
- [44] Clarke R, Smith AD, Jobst KA, Relsum H, Sutton L, Ueland PM (1998) Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. *Arch Neurol* **55**, 1449-1455.
- [45] Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, Wilson PW, Wolf PA (2002) Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med* **346**, 476-483.