

The Effects of the Calcium Binding Protein Apoaequorin on Memory and Cognitive Functioning in Older Adults

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Abstract

We report here on a randomized, double-blind, placebo-controlled study designed to assess the effect of an apoaeguorin supplement on specific areas of cognitive function using quantitative, computerized cognitive assessment tools. Changes in cognitive function, which occur as an individual ages have been described as having significant impacts on an individual's quality of life. Decreases in cognitive ability may result in reduced social and intellectual interaction. This study examined community dwelling, older adults taking either an apoaeguorin supplement or a matched placebo for 90 days. Apoaeguorin is a calcium-binding protein originally isolated from the jellyfish *Aequorea victoria*.

Methods

A sample of 218 adults, aged 40 to 91 years, with self-reported memory concerns were randomly assigned to receive either apoaeguorin (Experimental Arm) or a matched placebo (Control Arm).

Outcome Measure

Scores from a computerized cognitive test battery (CogState Ltd) performed pre- and post-treatment and at several time points during the study.

Results

There were no meaningful differences between the Experimental and Control Arms on any parameter at baseline. The Experimental Arm showed a statistically significant improvement in a number of different measures of cognitive function, including executive function, visual and verbal learning, and memory. Significant improvements were seen on a number of different tasks from the CogState Ltd. Research Battery. Apoaeguorin was very well-tolerated in this study.

Conclusion

These results indicated a strong relationship between apoaeguorin and improvements on a quantitative measure of cognitive function. Apoaeguorin is a well-tolerated supplement that has shown to improve cognitive function in aging adults. These results suggest potential utility for apoaeguorin to address the declines in cognitive function associated with aging.

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Introduction

Every seven seconds in the United States, another member of the baby boomer generation turns 65. As the baby boomer population has moved through the stages of their lives, they have been responsible for significant changes in society. Not surprisingly, as this generation has gotten older, they have become concerned about healthy aging and are committing resources to diminishing the changes that frequently occur with advancing age.

Normal cognitive function is central to many people's quality of life. Memory lapses such as forgetting where you put your keys or the contents of a grocery list are seen as a normal part of the aging process. Changes in an individual's cognitive function impact their ability to continue to live full, independent lives.

The deterioration of cognitive function frequently associated with aging is an area of great concern for many. Members of the baby boomer generation are not content to accept age-related cognitive decline as normal, they are seeking solutions, being proactive, and looking for ways to mitigate age-related cognitive decline.

Role of Calcium

Calcium has many functions throughout the body. It is vital to many aspects of physiology, including gene expression, cell death, learning, and memory. Calcium is a critical second messenger facilitating physiological responses to a variety of physical, chemical, or electrical stimuli.

Calcium levels in the nervous system are tightly regulated in most situations. During normal physiological activity, there is a brief (seconds to minutes) elevation in intracellular calcium concentration. This short-lived increase in the level of calcium quickly returns to baseline without any adverse effects on the neuron. As we age, the body's ability to closely regulate and maintain calcium levels is reduced. Elevated calcium levels have frequently been noted in older neurons, and aging nervous systems exhibit increased sensitivity to changes in calcium levels. An inability to maintain appropriate intracellular calcium levels is believed to be involved in brain aging.

The changes seen when cells are unable to regulate calcium levels gave rise to the calcium hypothesis of brain aging. First proposed by Khachaturian, the calcium hypothesis was based on effects noted in relation to elevated calcium levels in neuronal cells.^{1, 2, 3} Khachaturian postulated that small increases in the levels of intracellular calcium, resulting from a dysregulation of calcium homeostasis, could, over time lead to damage similar to that seen after an acute rise in calcium levels, eventually leading to cell death. Many investigators have postulated that sustained alterations in calcium levels provide a final, common pathway for age-associated alterations in brain function.⁴

Apoaequorin

Apoaequorin is a naturally occurring calcium-binding protein, originating from a species of jellyfish, which is similar in amino acid sequence to human calcium-binding proteins. Calcium-binding proteins have been shown to be vital in the regulation of calcium levels in certain cell types with lower levels present in aged cells. Studies have noted that cells that are exhibiting calcium dyshomeostasis exhibit decreased levels of calcium-binding proteins.⁵

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A possible strategy for managing altered calcium levels in neuronal cells involves supplementation with exogenous calcium-binding proteins. Apoaequorin has been shown in laboratory studies to regulate intracellular calcium levels and provide neuroprotection against ischemic cell death.⁶ Based upon its sequence similarity and the in vitro and in vivo results,⁷ we hypothesized that apoaequorin has the potential to improve the function of aging neurons. Increasing the levels of calcium-binding proteins have been shown to increase the control of intracellular calcium levels, resulting in appropriately regulated calcium levels which could affect the progression of age associated memory loss.

Study Design

The Madison Memory Study was a randomized, double-blind, placebo-controlled study designed to examine the effect of apoaequorin on cognitive function in older adults. Community dwelling participants were randomized into either the Experimental Arm (apoaequorin) or Control Arm (placebo) at a ratio of 3:2. Participants in the Control Arm received capsules containing only white rice flour. Participants in the Experimental Arm received capsules containing apoaequorin (10 mg) and white rice flour. Capsules were size and color matched. Participants were instructed to take one (1) capsule daily for the duration of the study.

The primary objective of the Madison Memory Study was to assess the effect of apoaequorin (10 mg daily) cognitive function. Quantitative computerized cognitive tests were utilized to examine the effect of apoaequorin over time and compared to placebo.

Secondary objectives for the Madison Memory Study included quality of life measures including, but not limited to, sleep quality, energy levels, and participants' reported quality of life. Widely used qualitative surveys were used to compare the Experimental and Control Arms.

Participants

The Madison Memory Study was comprised of 218 participants (148 females and 70 males) aged 40 to 91 ($\mu = 62.48$) years.

Inclusion Criteria:

- **Healthy males and females not excluded by predetermined exclusion criteria**
- **Age between 40 to 95 at Baseline/Day 0 test**
- **Concerns related to memory issues**
- **Ability to comply with study protocol and complete periodic computerized cognitive tests**

Exclusion Criteria

- **A history of uncontrolled hypertension**
- **Untreated psychotic or major depressive disorder**
- **A significant neurological disease**
- **The inability to adhere to study protocol or complete periodic computerized cognitive tests**

	<i>n</i>	<i>Age</i>	μ	<i>SD</i>
Group Characteristics	218 (70 male)	40 - 91	62.48	11.43
Experimental Arm	126 (43 male)	40 - 90	62.54	11.79
Control Arm	92 (27 male)	40 - 91	62.39	10.98

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Methods

All participants in the Madison Memory Study completed the AD8 Screening Interview (AD8) prior to baseline testing. Participants were required to complete five (5) computerized cognitive testing sessions. Computerized tasks were selected from the CogState Research Battery (CogState Ltd.). The CogState Research Battery was administered by trained proctors at predetermined testing intervals. The primary efficacy variable was change from Baseline/Day 0 to Day 90 on tasks that selected from the CogState Research Battery. Measurements recorded for analysis include speed of performance, total number of correct moves per second, total errors, and the accuracy of performance.

Data Analysis

Data was analyzed using the IBM Statistical Package for the Social Sciences (SPSS) version 19 (IBM, Inc.). Data from CogState testing was analyzed as recommended by CogState Ltd., and previous reports.^{8, 9, 10, 11} Briefly, cognitive assessments were analyzed using paired and independent t-tests, and the Mixed-Model Repeated Measures Analysis of Covariance (ANCOVA). The Baseline/Day 0 scores served as the covariate for the Mixed-Model Repeated Measure ANCOVA. In addition to the entire study population, participants were segregated into analysis groups based on self-reported levels of cognitive impairment as measured by the AD8. Qualitative survey data was analyzed to determine descriptive statistics, using the Mann-Whitney U Test and the Wilcoxon Signed Rank Test. Group means and standard deviations were found for each cognitive assessment.

Effect Size

Effect size is a statistical measure that describes the strength or magnitude of the difference between two groups. Effect size was calculated by taking the difference in the means between two groups or time points and dividing that number by the pooled/combined standard deviation. The effect size value measures the strength of the observed effect between the two groups.

The effect size for Cohen's d is measured on a standardized scale starting at 0.0 and examines the relationship between two groups to determine the degree of difference between data scores. A Cohen's d of .25 indicates a weak effect of the experimental variable in relation to the variability in the sample from the Baseline to the Day 90 testing point. An effect size of .2 is considered a Small Effect; an effect size of .5 is a Medium Effect; and .8 or greater is considered a Large Effect.

AD8

To segregate participants by their level of self-reported cognitive impairment, a baseline score on the AD8 screening tool was acquired. The AD8 is a brief (eight-question), reliable screening tool which is sensitive and predictive in classifying non-demented or cognitively normal older adults from those with some level of cognitive impairment.^{12, 13} An AD8 score of 2 was used as a cut-off value to discriminate between cognitively normal and individuals with some level of cognitive impairment.¹⁴

Cognitive Measurement Tasks/Tools

The cognitive measures used in this study are part of the CogState Research Test Battery and have been described previously elsewhere.^{15, 16} CogState is a widely used neuropsychological battery of computerized cogni-

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tive tests that are an adaptation of standard neuropsychological tests. CogState's tasks were selected because they are brief, repeatable, have shown little or no practice effects, and have utility for repeated assessment of cognition in older adults. CogState's tasks are a selection of card games, mazes, and lists of common items. Practice sessions are presented before each task in sufficient number to ensure that the participant is aware of the rules for each task.

The CogState tasks used in this study were the Groton Maze Learning (GML), Groton Maze Recall (GMR), One Card Learning (OCL), Two Card Back (TWOB), One Card Back (ONB), Detection (DET), and Identification (IND) tasks.

The Detection (DET) task is a simple reaction time test that measures psychomotor speed. In this task, the participant must press the "Yes" key as quickly as possible when a card presented in the center of the screen turns face-up. The task ends when 35 correct trials were recorded. Mean speed of performance for correct responses is the outcome measure.

The Identification (IDN) task is a choice reaction time test that measures visual attention. In this task, the participant must press the "Yes" key as quickly as possible when the presented card is red or "No" if it is black. The task ends when 30 correct trials are recorded. Mean speed of performance for correct responses is the outcome measure.

The One-Card Learning (OCL) task assesses visual attention and recognition memory. In this task, participants are asked to respond "Yes" if the face-up card appeared previously in the task and "No" if it had not. Six cards were repeated in a total of 42 cards. Mean accuracy is the outcome measure.

The One-Back (OBK) test assesses visual attention and working memory. In this task, participants are asked to respond "Yes" if the face-up card was exactly the same as the card that immediately preceded it or "No" if it was not this card. The task ends when 30 correct trials are recorded. Mean speed of performance for correct responses is the outcome measure.

The Two Back (TWOB) test assesses visual attention and delayed recall. In this task, participants are asked to respond "Yes" if the face-up card was exactly the same as the card that was shown two cards earlier. The task ends when 30 correct trials are recorded. Mean speed of performance for correct responses is the outcome measure. The Two Back task is a version of the n-back continuous performance task for measure in attention and working memory first described by Kirschner in 1958.¹⁷

The Groton Maze Learning Test (GML) and the Groton Maze Recall (GMR) assess executive function and visual-spatial memory/problem solving.¹⁸ Executive function is comprised of those high-level cognitive processes, which help individuals' complete complicated tasks and accomplish goals. Executive function refers to mental skills that are coordinated in the frontal lobe and include the ability to manage time and attention, switch focus, plan and organize, remember details, and integrate past experiences. Compromised executive functioning has been strongly linked to the decreased ability to perform Instrumental Activities of Daily Living (IADL).¹⁹

In the GML and GMR, a 10x10 grid of tiles is presented on the computer screen. Within this grid is a 28-step hidden pathway. Starting at the top, left-hand corner, subjects are instructed to move through the maze one step

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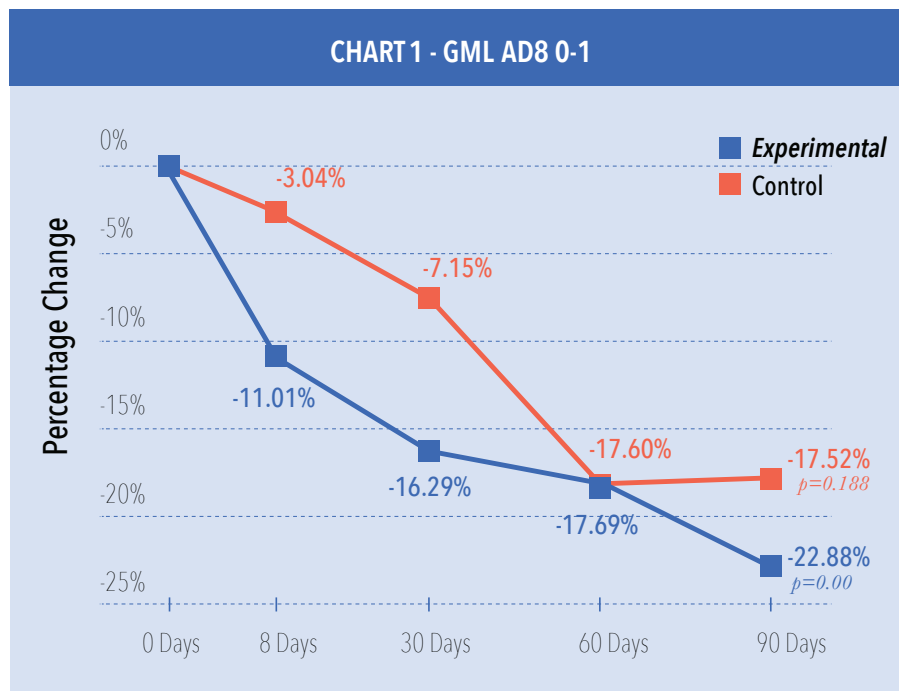
at a time in order to learn the correct pathway. The last tile in the maze is in the lower, right hand corner. Subjects are guided by audio and visual feedback. Subjects completed the GML five (5) times in succession during each testing session. The GMR repeats the same hidden maze seen earlier in the testing session. This round is presented approximately 30 minutes after the first five (5) rounds. The primary measure for both the GML and the GMR was the total number of errors, lower scores indicating better performance.

RESULTS

Groton Maze Learning (GML)

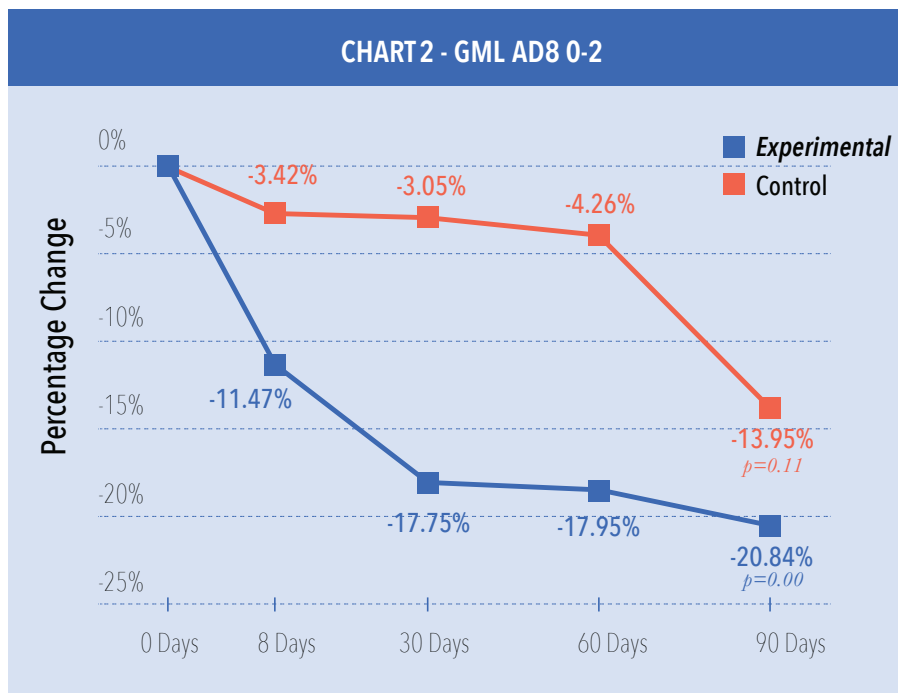
The Groton Maze Learning Tasks (GML and GMR) were used to examine the effect of the experimental substance on executive function as compared to the effect of the placebo on the Control Arm. The primary measurement used to score the GML is the total number of errors made during five (5) repetitions of a particular maze. The primary scoring measure for the GMR is the number of errors made repeating the same maze approximately 30 minutes later. A reduction in the number of errors is a measure of improvement on the GML and GMR.

When the entire study sample was analyzed, a significant difference between the Control and the Experimental Arms was not seen. However, when study participants with AD8 scores of 0-1 were analyzed a significant difference was seen in the Experimental Arm between Baseline/Day 0 and Day 90 ($p < 0.001$). No significant difference was seen between the Baseline/Day 0 and Day 90 scores for the Control Arm. The Experimental Arm showed a 23% reduction in total errors compared to the number of errors at Day 0. This represents a 31% greater reduction in the total errors for the Experimental Arm than was seen for the Control Arm. The Experimental Arm had a Cohen's d effect score of 1.0 indicating a Large Effect for the experimental substance. **(Chart 1)**



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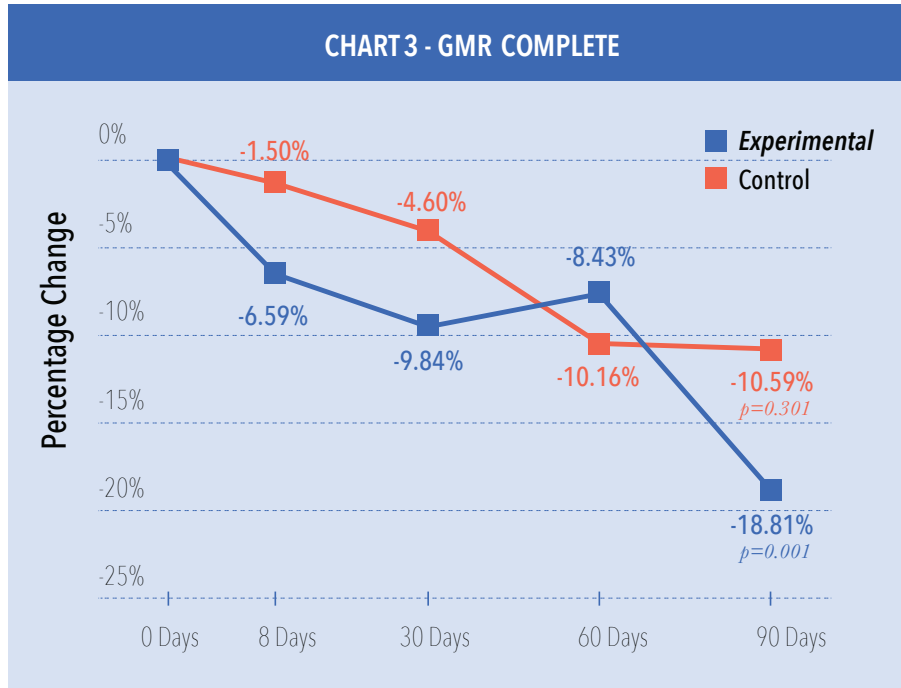
When results from participants with AD8 scores between 0-2 were analyzed, the change in the total errors in the Experimental Arm was significantly different when compared to Control Arm at each and every time point during the study compared to Day 0 with p values between 0.000 and 0.002. The Experimental Arm exhibited an almost 21% reduction in the total number of errors comparing Day 90 to Day 0. A Cohen's d effect size of .73 was calculated indicating a moderate to large effect for the experimental substance in reducing the total number of errors. (Chart 2)



Groton Maze Recall (GMR)

The effect of the experimental substance on executive function was more striking on the Groton Maze Recall task. Examining the entire study population, a statistically significant reduction in total errors of 18.81% was seen in the Experimental Arm ($p=0.001$). The Experimental Arm also showed an almost 80% greater reduction in total errors compared to the Control. An effect size of .46 was calculated suggesting that apoaequorin had a Moderate Effect. (Chart 3)

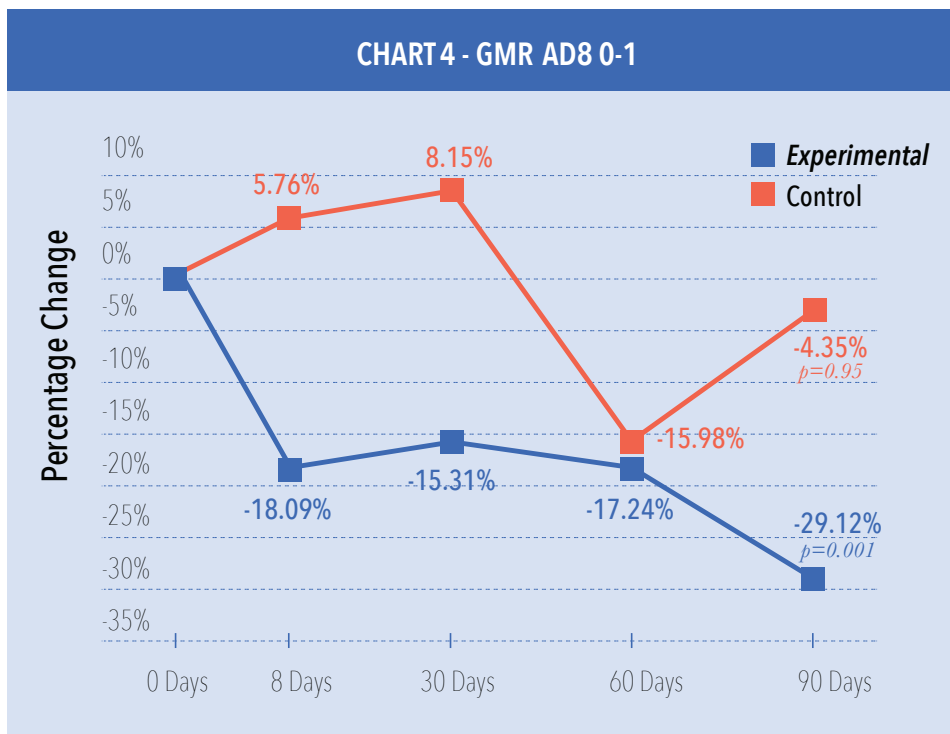
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When the results on the GMR for AD8 0-1 cohort were analyzed a significant difference was seen between the Experimental and Control Arms for the total number of errors. $F_{1, 54} = 7.19$, $p < .01$. Additionally there was a significant effect comparing the Experimental Arm at Baseline/Day 0 and Day 90, ($\mu = 8.92$, $SD = 20.33$), $t_{36} = 3.67$, $p < .001$, Cohen's $d = 0.8$.

The total errors in the Experimental Arm between Baseline/Day 0 and Day 90 decreased by 29.12% on the GMR compared to only 4.35% in the Control Arm. The effect size (-0.8) for the Experimental Arm indicates a Large Effect for apoaequorin in reducing the total number of errors. **(Chart 4)**

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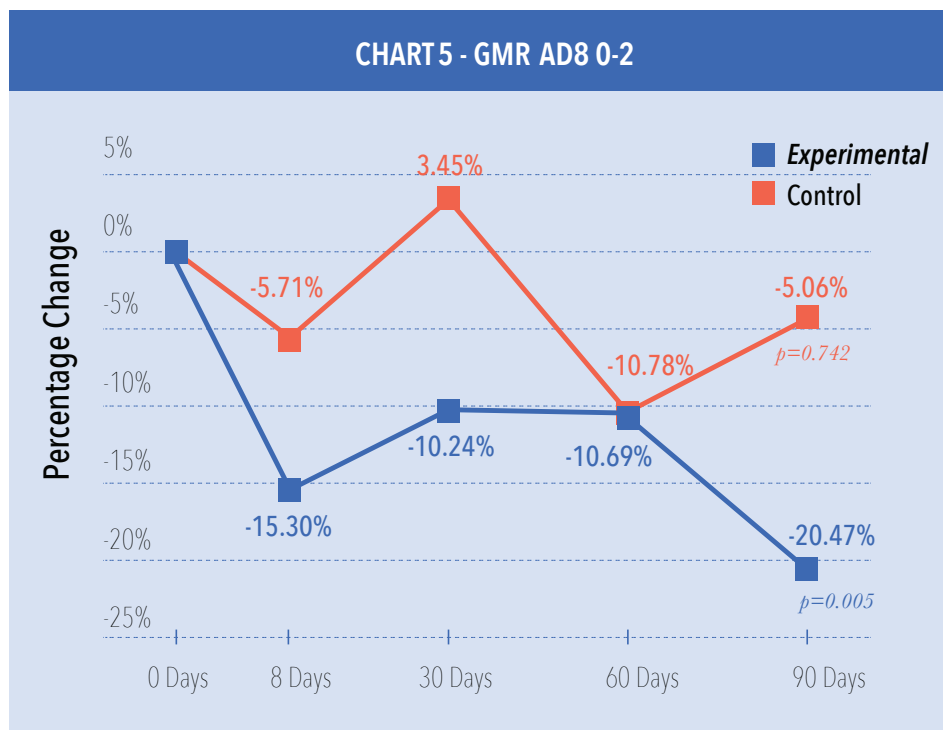


A significant difference in the total number of errors between the Experimental and Control Arms for participants who were considered within the range of normal to mild cognitive impairment (0- 2) on the AD8, $F_{1, 90} = 4.22$, $p < .05$. A significant effect was seen within the Experimental Arm from Baseline/Day 0 to Day 90, $p < .01$, with total errors decreasing by 20.47% and a medium effect size of -0.39. The Control Arm did not exhibit a significant effect comparing Day 90 to Day 0 $p=0.74$, with a reduction of total errors over time of 5%. **(Chart 5)**

The ability of apoaequorin to improve performance on the GMR was maintained for participants with AD8 scores between 0 and 3. A significant effect was seen in the Experimental Arm from Baseline/Day 0 to Day 90, $p < .006$, with total errors decreasing by 18.18% and a Medium Effect size of -0.43. The Control arm did not show significance comparing Day 90 to Day 0, with a reduction of total errors over time of 7.2%.

While significance was not noted on the complete study population for the Groton Maze Learning task, on individuals with self-reported minimal or no cognitive impairment, significant reduction in total error numbers and moderate to Large Effect size were seen. The Groton Maze Recall task demonstrated statistically significant reductions in total errors for the entire study population as well as a cognitively normal and cognitively normal to mild cognitive dysfunction subset. The Experimental Arm also showed Moderate to Large Effect sizes, which were not seen in the Control Arm.

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One Card Learning/Identification

Attention is part of cognitive function that is essential for learning and memory. Whatever people pay attention to (mentally) moves into working memory. Information that does not meet the attention threshold typically disappears from the memory system. Attention also involves directing the appropriate sensory receptors (eyes, ears, fingertips, etc.) and one's mind toward whatever needs to be learned and remembered. Individuals have a limited attention capacity, and only a small amount of information moves on to working memory. Attention was measured with several different card based task in the CogState Research Battery including the OCL, ONB, IDN, and DET tasks.

The One Card Learning (OCL) test measured the proportion of correct responses during the entire duration of the test. Participants were required to remember each card that was shown in addition to responding correctly, as to whether a card was new or had been seen previously.

A significant difference was seen for the complete study population in the Experimental Arm with a significant change from Baseline/Day 0 to Day 90, ($\mu = -0.03$, $SD = 0.14$), $t_{126} = -2.08$, $p < .05$. 61.47% of participants showed an improvement from the Baseline/Day 0 to Day 90 as measured by the accuracy of performance. The accuracy of performance is calculated by taking the arcsine transformation of the square root of the proportion of correct responses.

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A significant difference was also seen in the Experimental Arm for participants with AD8 scores of 2-5. The Experimental Arm saw a significant change from Baseline (Day 0) to Day 90 ($p < 0.05$). No significant changes were seen in the Control Arm.

Identification

The Identification task (IDN) measured the participant's accuracy as to whether a particular card had previously been seen. A significant difference was seen in the Experimental Arm between Day 0 and Day 90 in the AD8 0-1 sample ($p < 0.001$). Experimental Arm participants show a 47% improvement in their accuracy of performance at Day 90 as compared to Day 0. No significant difference was seen between Day 0 and Day 90 for the Control Arm.

Two Card Back

Working memory refers to the temporary storage and manipulation of information necessary for cognitive tasks such as language comprehension, learning, and reasoning. Working memory involves the simultaneous storage and processing of information. Working memory permits an individual to stay focused on a task, and keep thought in mind through distractions. Working memory is crucial to actively participating in discussions, being able to perform what you are planning to do, organizing materials and activities, and managing important financial activities.

Examining results from the Two Card Back task, participants in the Experimental Arm with AD8 scores of 0-1 exhibited a significant improvement in the ability to remember whether a presented card was the same card, which they had seen two cards previously. Significant improvements were seen at all but one testing point for the Experimental arm. The Control arm did not show a significant improvement for the Two Cards back task at any testing point. (Table 3)

Adverse Events

The Experimental and Control substances were very well-tolerated. Two participants experienced adverse events during the study. Each arm had a single adverse event and there were no serious adverse events (SAEs) in the study. Both participants were withdrawn from the study.

Discussion

There have been many reports on research examining the effect of a wide range of interventions focused on maintaining or improving cognitive function. The aging of the baby boomer cohort and the issues that occur with aging have increased interest in potential interventions that may positively reduce the "consequences of aging."

This study was designed to examine the effect of oral supplementation of the calcium-binding protein, apoaequorin on cognitive function in a study population selected from community dwelling older adults with self-reported cognitive difficulties or concerns. This randomized, double-blind, placebo-controlled study was performed to validate anecdotal reports of improvements in cognitive function after use of an oral apoaequorin dietary supplement. Changes in cognitive function were quantitatively assessed using tasks from the CogState Research Battery, which has shown the ability to quantitatively measure alterations in cognitive function.

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Our data showed statistically significant changes in a number of different measures of a range of cognitive functions in the Experimental Arm for the complete study population and the study subsets (AD8 0-1 or AD8 0-2) with little or no self-reported cognitive issues. This data supports the hypothesis that oral supplementation with a calcium binding protein, apoaequorin, could positively impact cognitive function.

The improvements seen for the study subset with no or minimal self-reported cognitive difficulties showed Cohen's effects size in the Medium to Large range, further indicating that the experimental substance was providing meaningful changes in cognitive function to that population.

We believe that additional studies with a more defined study population (AD8 0-1/2) would provide even clearer evidence for the ability of the calcium-binding protein apoaequorin to positively impact cognitive function in aged individuals with minimal or no cognitive impairment. With regard to the statistically significant results seen for populations with greater self-reported cognitive impairment at Day 0, a larger study and a longer time frame may provide additional evidence of a beneficial effect in this particular population.

It is highly unlikely that the results seen on these tasks are due to chance. The differences seen in the Experimental Arm showing improvement for a variety of cognitive functions were not trivial and in many cases represent a difference of greater than 10% or 15% respectively from baseline scores. The differences between Day 90 and baseline values for the Experimental Arm were also highly statistically significant while no significance was seen in the Control Arm.

The strengths of the Madison Memory Study include the recruitment of a community dwelling population and the use of quantitative cognitive testing. The CogState Research Battery provided the ability to quantify subtle changes in cognitive performance in a population of older individuals with varied degrees of cognitive impairment without learning or ceiling effects.^{20, 21}

Conclusion

Apoaequorin is a well-tolerated, nontoxic calcium-binding protein that has demonstrated the ability to improve cognitive function. Daily use of an oral apoaequorin-containing supplement/capsule had a statistically significant effect and demonstrated improvements for a number of different cognitive functions learning in a population of older individuals with little or no self-reported cognitive impairment. These results indicated a strong relationship between apoaequorin and improvements on a measure of cognitive function, specifically verbal learning. These results imply possible utility for apoaequorin to reduce declines in cognitive function associated with aging.

References

1. Khachaturian ZS, Cotman CW & Pettegrew W. (1989) Calcium, membranes, aging and disease. *Annals of the New York Academy of Sciences*, 568:1-292.
2. Khachaturian ZS (1991) Calcium and the aging brain: upsetting a delicate balance? *Geriatrics*, 46:78-79.
3. Khachaturian ZS (1994) Calcium hypothesis of disease and brain aging. *Annals of the New York Academy of Sciences*, 747:1-11.

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4. Khachaturian ZS (1987) Hypothesis on the regulation of cytosol calcium concentration and the aging brain. *Neurobiol. Aging*, 8:345-346.
5. Detert JA, Hochstetter EL, Van Langendon TM, & Moyer JR. (2011) Time course and effectiveness of apoaequorin as a neuroprotectant in the brain. *Society for Neuroscience Abstracts*, Program No. 781.04.
6. Detert JA, Heisler JD, Hochstetter EL, Van Langendon TM, & Moyer JR. (2009) Neuroprotection of hippocampal CA1 neurons from ischemic cell death using the calcium-binding protein aequorin. *Society for Neuroscience Abstracts*, Program No. 52.24
7. Detert JA, Schmidt M, Kampa N, Tao P, & Moyer JR. (2007) Apoaequorin protects adult and aging hippocampal CA1 neurons from ischemic cell death. *Society for Neuroscience Conference*, San Diego, CA.
8. Cargin JW, Maruff P, Collie A, et al. (2007) Decline in verbal memory in older adults. *Journal of Clinical and Experimental Neuropsychology* 29:(7)706-718.
9. Collie A, Maruff P, Makdissi M, McStephen M, Darby DG, and McCrory P. (2004) Statistical procedures for determining the extent of cognitive change. *British Journal of Sports Medicine* 38:273-278.
10. Pietrzak RH, Fredrickson A, Snyder PJ, and Maruff P. (2010) A comparison of statistical approaches used to evaluate change in cognitive function following pharmacologic challenge. *Human Psychopharmacology: Clinical and Experimental* 25:335-341.
11. Fredrickson A, Snyder PJ, Cromer J, Thomas E, et al. (2008) The use of effect sizes to characterize the nature of cognitive change in psychopharmacological studies. *Human Psychopharmacology: Clinical and Experimental* 23:425-436.
12. Galvin JE, Roe CM, and Morris JC. (2007) Evaluation of cognitive impairment in older adults: combining brief informant and performance measures. *Arch Neurol.* 64:718-724.
13. Galvin JE, Roe CM, Coats MA, Morris JC. (2007) Patient's rating of cognitive ability: using a brief informant interview, as a self-rating tool. *Arch Neurol.* 64:725-730.
14. Galvin JE, Fagan AM, Holtzman DM, Mintun MA, Morris JC. (2010) Relationship of screening tests with biomarkers. *Brain* 133:3290-3300.
15. Fredrickson J, Maruff P, Woodward M, et al. (2010) Evaluation of the usability of a brief computerized cognitive screening test in older people for epidemiological studies. *Neuroepidemiology.* 34:64-75.
16. Maruff P, Thomas E, Cysique L et al. (2009) Validity of the CogState Brief Battery: relationship to standardized tests and sensitivity to cognitive impairment. *Arch Clin Neuropsychol.* 24:165-178.
17. Kirchner WK. (1958), Age differences in short-term retention of rapidly changing information. *Journal of Experimental Psychology*, 55(4), 352-358.
18. Pietrzak RH, Maruff P, Mayes LC, Roman SA, Sosa JA, and Snyder PJ. (2008) An examination of the construct validity and factor structure of the Groton Maze Learning Test, A new measure of spatial working memory, learning efficiency and error monitoring. *Archives of Clinical Neuropsychology.* 23:433-445.
19. Marshall GA, Rentz DM, Frey MT, Locascio JJ et al. (2011) Executive Function and instrumental activities of daily living. 7:300-308.
20. Collie A, Maruff P, Darby DG, McStephen M. (2003) The effects of practice on the cognitive test performance of neurologically normal individuals assessed at brief test-retest intervals. *J. Inter. Neuropsychol. Soc.* 9:419-428.
21. Maruff O, Falsetti MG, Collie A, Darby D, McStephen M. (2005) Fatigue-related impairment in the speed, accuracy and variability of psychomotor performance: comparison with blood alcohol levels. *J Sleep Research*, 14:21-27.

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