

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

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Background

We report here on a double-blind, placebo controlled study designed to assess the effect of an apoeaquorin supplement, compared to placebo, on specific areas of cognitive functioning using quantitative, computerized assessments. In a recent double-blind, placebo controlled trials, older adults taking an apoeaquorin supplement reported improvements in cognitive functioning. Apoeaquorin, a calcium-binding protein, is a unique active ingredient originally isolated from the jellyfish *Aequorea victoria*.

Methods

A total of 218 adults, aged 40 to 91 years, who had self-reported memory concerns were randomly assigned to receive a 90 day supply of either apoeaquorin 10 mg daily or a matched placebo. Participants were tested at predetermined time points using computer-based assessments from CogState Ltd (www.cogstate.com). Changes on specific assessments of cognitive function were measured at various time points during the study.

Results

The apoeaquorin arm showed a significant improvement in scores of executive functioning on the Groton Maze Learning task over the 90 day study period. In measures of learning, study participants given apoeaquorin demonstrated significantly improved recall performance at Day 90 compared to Baseline. In cognitively normal participants, segregated by the level of cognitive impairment reported at Baseline on the AD8 Dementia Screening Interview, a significant reduction in the number of errors made on the Groton Maze Delayed Recall task was seen compared to placebo. In an assessment of short-term memory and learning, the apoeaquorin arm saw significant improvement at Day 90 compared to baseline in the One Card Learning task. The apoeaquorin group also improved their ability to recall shopping list items from a previously presented list in the International Shopping Recall List test. Apoeaquorin was very well tolerated in this study.

Conclusion

These results indicated a strong relationship between apoeaquorin and improvements on several quantitative measures of cognitive function. Apoeaquorin has been shown to be a well-tolerated and effective supplement for use by adults as they age. These results suggest an important role and potential therapeutic utility for apoeaquorin in delaying or modifying the decline in cognitive functioning associated with aging.

Introduction

Alzheimer's disease (AD) is the most common type of dementia and currently affects approximately 5.4 million Americans. Research has connected some forms of AD to heredity, however, the majority of AD cases are spontaneous in occurrence and individuals are at greater risk for developing AD as they age¹.

Now the sixth leading cause of all deaths in the U.S., Alzheimer's disease continues to rise dramatically. This phenomenon is unlike many other highly fatal illnesses such as heart disease, stroke and prostate cancer. Preliminary data reported in the 2011 edition of "Alzheimer's Disease Facts and Figures" indicate that between the years 2000 and 2008 stroke deaths decreased by 20 percent and heart disease deaths decreased by 13 percent. Deaths caused by AD, on the other hand, increased by 66 percent in that eight-year period.

Mild cognitive impairment (MCI) is a stage between normal forgetfulness associated with aging and the onset of dementia or Alzheimer's disease. Those affected by MCI have difficulty thinking and memory but are not prevented from everyday activities. Unlike Alzheimer's, those with MCI are aware of their forgetfulness. People with MCI progress to dementia at a higher rate than those with no memory impairment. Detecting MCI and implementing measures to potentially alter its course may be the best strategy for addressing the rapidly growing incidence of Alzheimer's disease.

Current Strategies

There are currently two types of treatment available for the management and treatment of AD. Each type works differently in the brain and targets a different pathway involved in neuron function, yet neither type of treatment represents a cure. Acetylcholinesterase inhibitors work by helping to increase the amount of acetylcholine in the brain, a chemical that is important for memory and learning. Currently there are three drugs in this class: donepezil, rivastigmine, and galantamine.

The other class of medications are called NMDA (N-Methyl-D-aspartate) receptor antagonists, or glutamate pathway modifiers. They work by interfering with the neurotransmitter glutamate, a molecule that normally targets the NMDA receptor and activates it by binding the receptor. Activation of the NMDA receptor through binding it sets off a cascade of reactions and signals in brain cells that, like the acetylcholine pathway, are critical in the processes of learning and memory. By targeting NMDA receptors and binding to them in brain cells, NMDA receptor antagonists block the activity of glutamate. Glutamate is considered a culprit in AD patients because when present at excessive levels it over stimulates and effectively kills off brain cells.

The only drug in this class currently approved to treat the cognitive symptoms of moderate to severe AD is memantine. Some studies have provided promising evidence that memantine is effective in treating moderate to severe AD. However, a report published in the online edition of "Archives of Neurology" in April 2011 dashed many hopes with the news that Namenda appears to be ineffective in treating individuals with mild stages of AD.

Role of Calcium

Calcium is vital to many aspects of brain physiology, including growth, learning and memory and serves as a critical intracellular messenger. In response to stimulatory signals from the environment, both electrical and chemical in nature, calcium ions flow through specialized channels in the plasma membrane of cells and then communicate with neurotransmitters to generate an appropriate response to the original signal. The integral relationship between calcium dysregulation and the deterioration of memory and cognitive functioning suggests the regulation of cytosolic calcium as a potential therapeutic approach to the treatment of age related memory loss and Alzheimer's disease³.

Cells maintain a low level of cytosolic calcium through the use of calcium-binding proteins and calcium ATPase pumps². Age related increases in cytosolic calcium levels are connected to major factors and contributors of AD, such as amyloid precursor protein mutations, Apoε4 expression, calcium homeostasis modulator 1 mutations, Aβ plaques, tau hyperphosphorylation, apoptosis and synaptic dysfunction¹.

Unique Approach

A novel strategy for controlling unregulated calcium levels in neuronal cells involves calcium-binding proteins. Studies have demonstrated that in cells with decreased levels of calcium-binding proteins, calcium homeostasis is adversely affected. This class of proteins has been shown to be vital in the regulation of calcium levels in certain cell types and is naturally depleted during the aging process. If intracellular calcium levels could be more tightly controlled through the activity of calcium-binding proteins, it is possible that neurodegenerative diseases like AD might be more effectively treated.

Apoaequorin is a calcium binding protein, originating from a species of jellyfish but similar in amino acid sequence to human endogenous calcium-binding proteins which are depleted in aging. Apoaequorin has been shown in laboratory studies to regulate intracellular calcium levels and serve as a neuroprotectant against ischemic cell death⁴. The following reports on the effects of Apoaequorin on memory and cognitive functioning in the Madison Memory Study.

Methods

All participants completed the AD8 Dementia Screening Interview (AD8) prior to their baseline cognitive testing. Participants were additionally required to complete five (5) cognitive testing sessions. The CogState Research Battery (CogState Ltd.) was utilized and included measures of executive function, speed of visual processing, psychomotor function, attention, verbal learning, delayed recall, and simple and complex working memory. Cognitive testing sessions lasted between 35 to 60 minutes depending on the participant, and were administered by a trained researcher at predetermined intervals throughout the three (3) month study.

The primary efficacy variable measured the change from the Baseline/Day 0 to Day 90 as recorded by the CogState software. The measurements included speed of performance, total number of correct moves per second, total errors, and the accuracy of performance. The secondary efficacy variables were self-reported improvements defined by positive changes from Baseline/Day 0 to Day 90 on the qualitative survey instruments.

Data Analysis:

Analysis was conducted using the IBM Statistical Package for the Social Sciences (SPSS) version 19 (IBM, Inc.). Data from all 218 participants were included in the analysis. The qualitative survey data were analyzed to find descriptive statistics, the Mann-Whitney U Test and the Wilcoxon Signed Rank Test. Group means and standard deviations were found for each cognitive assessment. Each cognitive assessment was analyzed using paired and independent t-tests, and the Mixed-Model Repeated Measures Analysis of Covariance (ANCOVA). The Baseline/Day 0 test scores served as the covariate for the Mixed-Model Repeated Measure ANCOVA. Participant data was segregated for analysis based upon self-reported level of impairment as measured by the AD8 score prior to Baseline/Day 0.

Participants:

The Madison Memory Study sample was comprised of 218 participants (148 females and 70 males) aged 40 to 91 ($\mu=62.48$) years. Eligible participants were randomized, by a 2:3 ratio, into the Placebo arm ($n=92$) or the Apoaequorin arm ($n=126$) respectively.

Inclusion Criteria:

- Healthy males and females that were not excluded by the predetermined exclusion criteria
- Age between 40 to 95 on Baseline/Day 0 testing
- Concerns related to memory difficulties
- Ability to comply with study protocol and complete periodic computerized cognitive testing

Exclusion Criteria:

- A history of uncontrolled hypertension
- Untreated psychotic or major depressive disorder
- A significant neurological disease
- Inability to adhere to study protocol or complete computerized testing

Methods

Participant Characteristics				
	<i>n</i>	Age Range	μ	<i>SD</i>
Group Characteristics	218 (70 male)	40 - 91	62.48	11.43
Apoaequorin arm	126 (43 male)	40 - 90	62.54	11.79
Placebo arm	92 (27 male)	40 - 91	62.39	10.98

Study Design:

The Madison Memory Study was a double-blind, placebo controlled study. Participants were randomized into the Control arm or the Experimental arm with a random number generator using a 2:3 ratio. In the Control, or Placebo arm, participants received a supply of capsules containing only white rice flour. Participants in the Experimental, or Apoaequorin arm, received a supply of capsules containing 10 mg of Apoaequorin in addition to the white rice flour. Placebo capsules were size and color matched to the Apoaequorin capsules. Participants were required to take one (1) capsule daily for the entire duration of the three (3) month study.

Primary Objective:

To compare and assess the effectiveness of Apoaequorin (10 mg daily) for improvement of memory and cognitive functioning.

Secondary Objective:

To assess the effectiveness of Apoaequorin in areas including, but not limited to, measures of sleep quality, energy levels, and participants' quality of life.

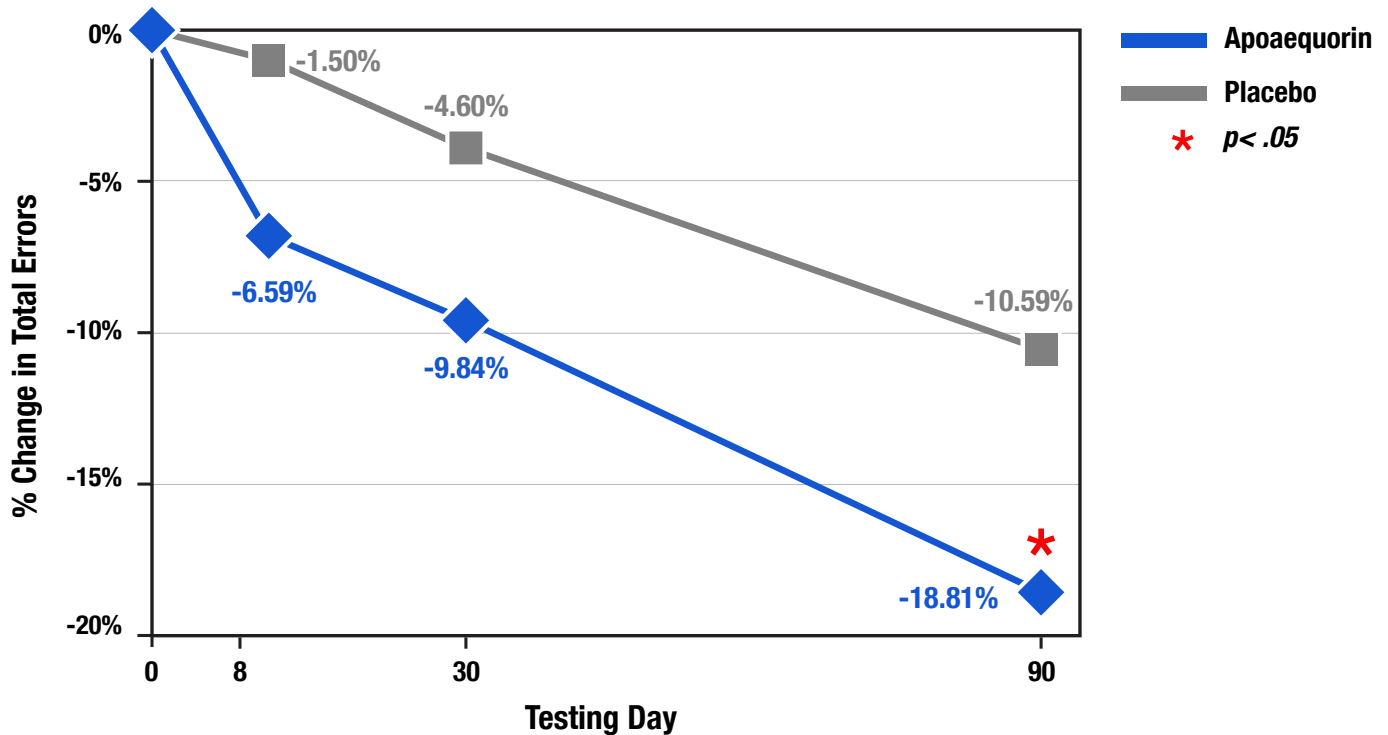
Results

The Groton Maze:

The Groton Maze Learning (GML) and the Groton Maze Recall (GMR) cognitive tasks measured participants' executive function and delayed recall. The GML task reported the total number of errors participants made repeating the same maze five (5) times. Whereas, the GMR task reported the total number of errors made during the participants' recall of the same maze seen previously in the GML.

- There was not a significant change over time between arms when looking at the complete study sample on the GML. However, there was a significant change over time between Baseline/Day 0 and Day 90 within the Apoaequorin arm, ($\mu= 1.69$, $SD= 5.63$), $t_{126}= 2.09$, $p < .001$, with a decrease in total errors by 18.81% (**Figure 1**).
- Additionally, participants in the Apoaequorin arm within the normal cognitive range (0 to 1) on the AD8 showed significant change over time on the GML from Baseline/Day 0 to Day 90, ($\mu= 12.81$, $SD= 18.47$), $t_{36}= 4.29$, $p < .000$, Cohen's $d= 1.00$. There was a decrease in total errors by 22.88% over the course of the study.

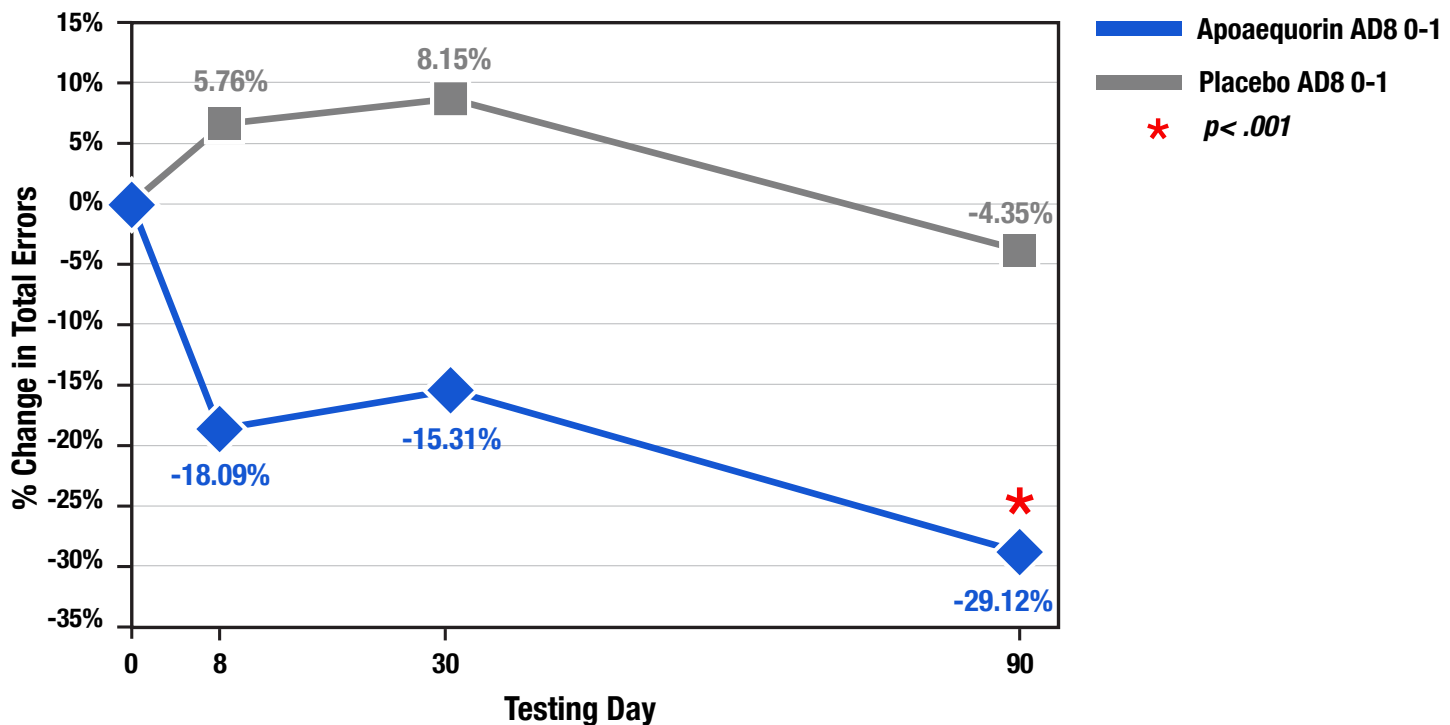
Figure 1: Groton Maze Learning



Results

- There was a significant difference over time in the total number of errors reported on the GMR between the Apoaequorin arm and the Placebo arm for participants who were considered within the range of normal to mild cognitive impairment (0 to 2) on the AD8, $F_{1, 90} = 4.22, p < .05$. A significant effect was seen within the Apoaequorin arm from Baseline/Day 0 to Day 90, ($\mu = 1.71, SD = 5.06$), $t_{57} = 2.57, p < .05$, with total errors decreasing on the GMR by 19.42%.
- A significant difference was seen over time between the Apoaequorin arm and the Placebo arm in the total number of errors made on the GMR for participants who scored within the normal cognitive range (0 to 1) on the AD8, $F_{1, 54} = 7.19, p < .01$. Additionally there was a significant effect seen within the Apoaequorin arm when comparing the Baseline/Day 0 and Day 90 results, ($\mu = 8.92, SD = 20.33$), $t_{36} = 3.67, p < .001$, Cohen's $d = 0.8$.
- The total errors in the Apoaequorin arm for participants who scored within the normal cognitive range (0 to 1) on the AD8, between Baseline/Day 0 and Day 90, decreased by 29.12% on the GMR compared to only 4.35% in the Placebo arm (**Figure 2**).

Figure 2: Groton Maze Recall



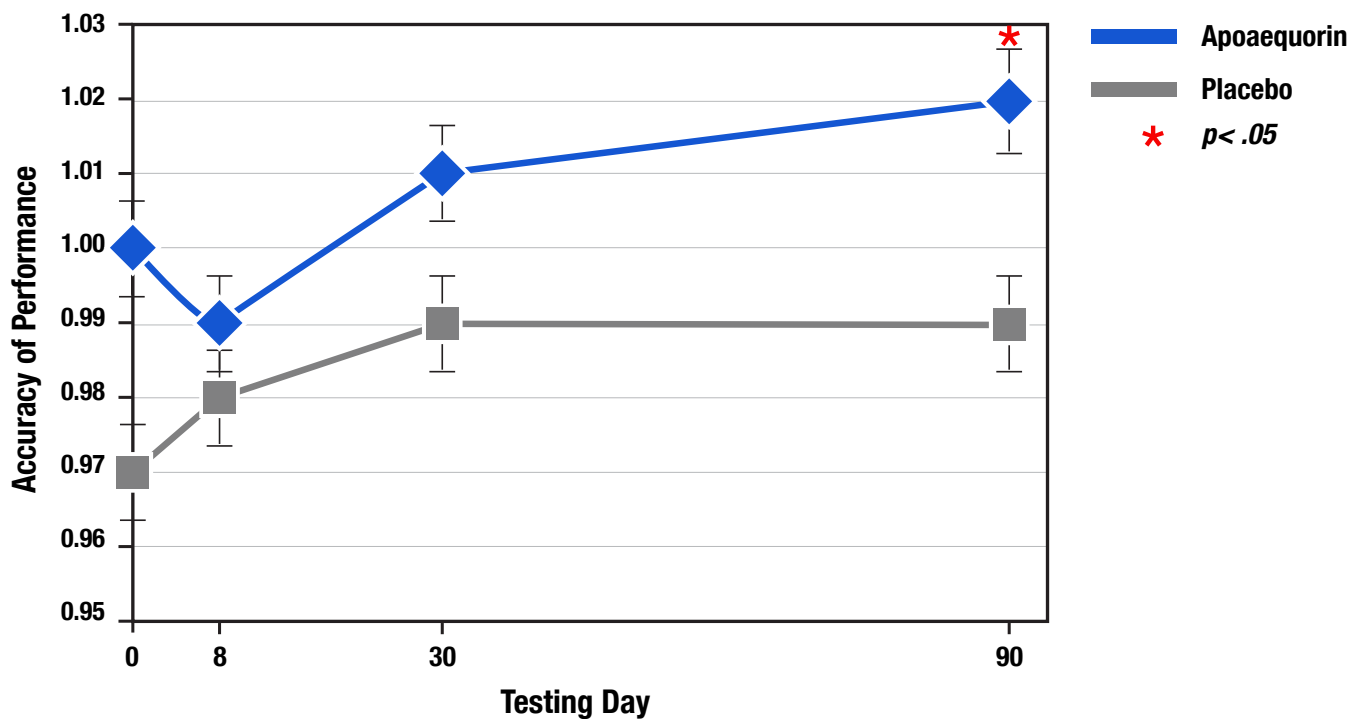
Results

One Card Learning:

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.

- The Apoaequorin arm saw a significant change from Baseline/Day 0 to Day 90, ($\mu = -0.03$, $SD = 0.14$), $t_{126} = -2.08$, $p < .05$, with 61.47% of participants showing an improvement from the Baseline/Day 0 to Day 90 as measured by the accuracy of performance (**Figure 3**). The accuracy of performance is the arcsine transformation of the square root of the proportion of correct responses.

Figure 3: One Card Learning



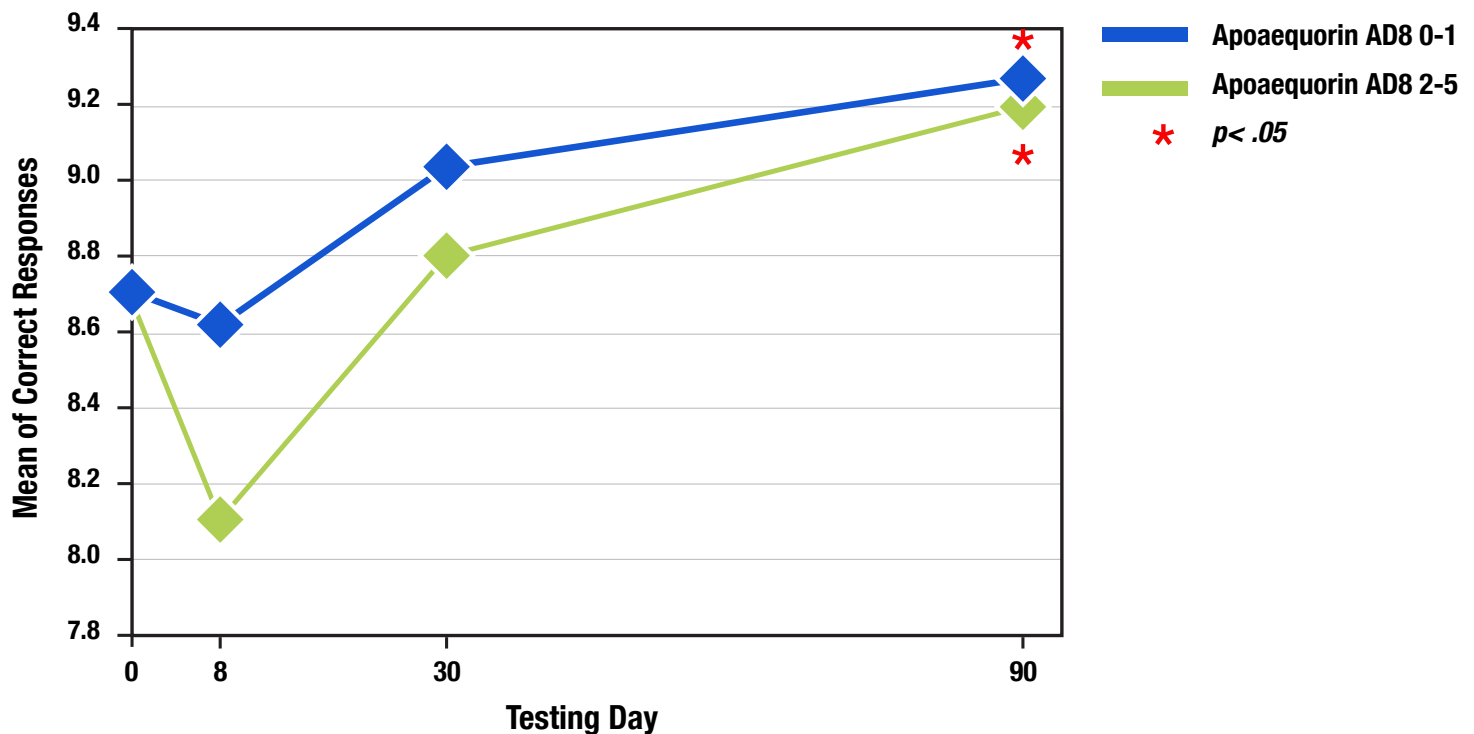
Results

The International Shopping Recall List:

The International Shopping Recall List (ISRL) measures the number of correct shopping list items the participant can recall from a previously presented list of items. Verbal learning and delayed recall are cognitive functions specifically measured by the ISRL.

- There was a significant difference over time between the total number of correct responses on the ISRL for participants who were within the normal cognitive range (0 to 1) on the AD8, ($\mu = -2.81$, $SD = 4.94$), $t_{36} = -3.46$, $p < .001$ (Figure 4).
- A significant effect was also seen over time on the ISRL for participants within the mild to moderate cognitive range (2 to 5) on the AD8 in the Apoaequorin arm, ($\mu = -2.76$, $SD = 5.41$), $t_{67} = 2.40$, $p < .05$ (Figure 4).

Figure 4: International Shopping Recall List



Summary

Overall, participants in the Apoaequorin arm and within the normal to mild cognitive range on the AD8 saw a significant positive change over the three (3) month study period in:

- Verbal Learning (ISRL)
- Memory (OCL)
- Delayed recall (GMR and ISRL)
- Executive Function (GML)

Participants taking Apoaequorin and who were considered to be within the moderate to severe cognitive range on the AD8 also saw improvements. Although these results were not significant, this data provides evidence that a study longer than 90 days may be necessary for individuals who suffer from greater cognitive impairment.

Conclusion

Dysregulation of cytosolic calcium occurs naturally for many in the aging process, and has been shown to be a common precursor for cognitive decline and Alzheimer's disease¹. Apoaequorin has been shown in laboratory studies to decrease cell death due to ischemia by 55% in aged hippocampal cells⁴. This study establishes a relationship between Apoaequorin and improvements on quantitative measures of cognitive function. Overall, the results suggest that Apoaequorin has an important role and potential therapeutic utility in delaying or modifying the decline in cognitive functioning associated with aging.

References

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