

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

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## Background

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We report here on a double-blind, placebo controlled study designed to assess the effect of an apoaequorin dietary supplement, compared to placebo, on specific areas of cognitive functioning using quantitative, computerized assessments. In recent open label and double-blind, placebo controlled trials, older adults taking an apoaequorin dietary supplement reported improvements in cognitive functioning. Apoaequorin is a protein originally isolated from the jellyfish *Aequorea victoria*.

## Methods

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

## Results

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The apoaequorin 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 apoaequorin demonstrated significantly improved recall performance at Day 90 compared to Baseline. In an assessment of short-term memory and learning, the PrevaGen arm saw significant improvement at Day 90 compared to baseline in the One Card Learning task. The PrevaGen group also improved their ability to recall shopping list items from a previously presented list in the International Shopping List test. Apoaequorin was very well tolerated in this study.

## Conclusion

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These results indicated a strong relationship between apoaequorin and improvements on several quantitative measures of cognitive function. Apoaequorin has been shown to be a well-tolerated and effective dietary supplement for use by adults as they age. These results suggest a supportive role for apoaequorin in improving memory for people experiencing normal age-related memory problems.

## Methods

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All participants completed the AD8 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

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<b>Participant Characteristics</b>				
	<i>n</i>	Age Range	$\mu$	<i>SD</i>
<b>Group Characteristics</b>	218 (70 male)	40 - 91	62.48	11.43
<b>Apoaequorin arm</b>	126 (43 male)	40 - 90	62.54	11.79
<b>Placebo arm</b>	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.

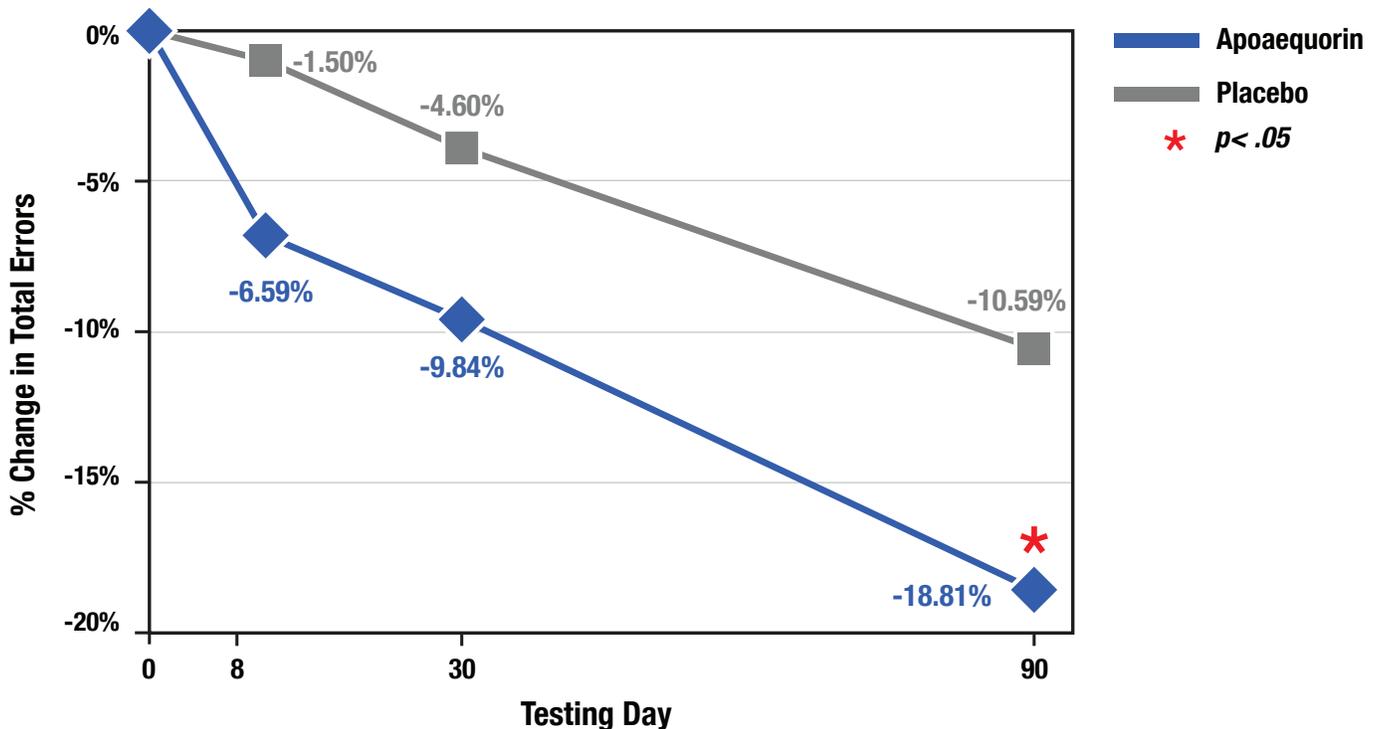
## Results

### 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**).

**Figure 1: Groton Maze Learning**

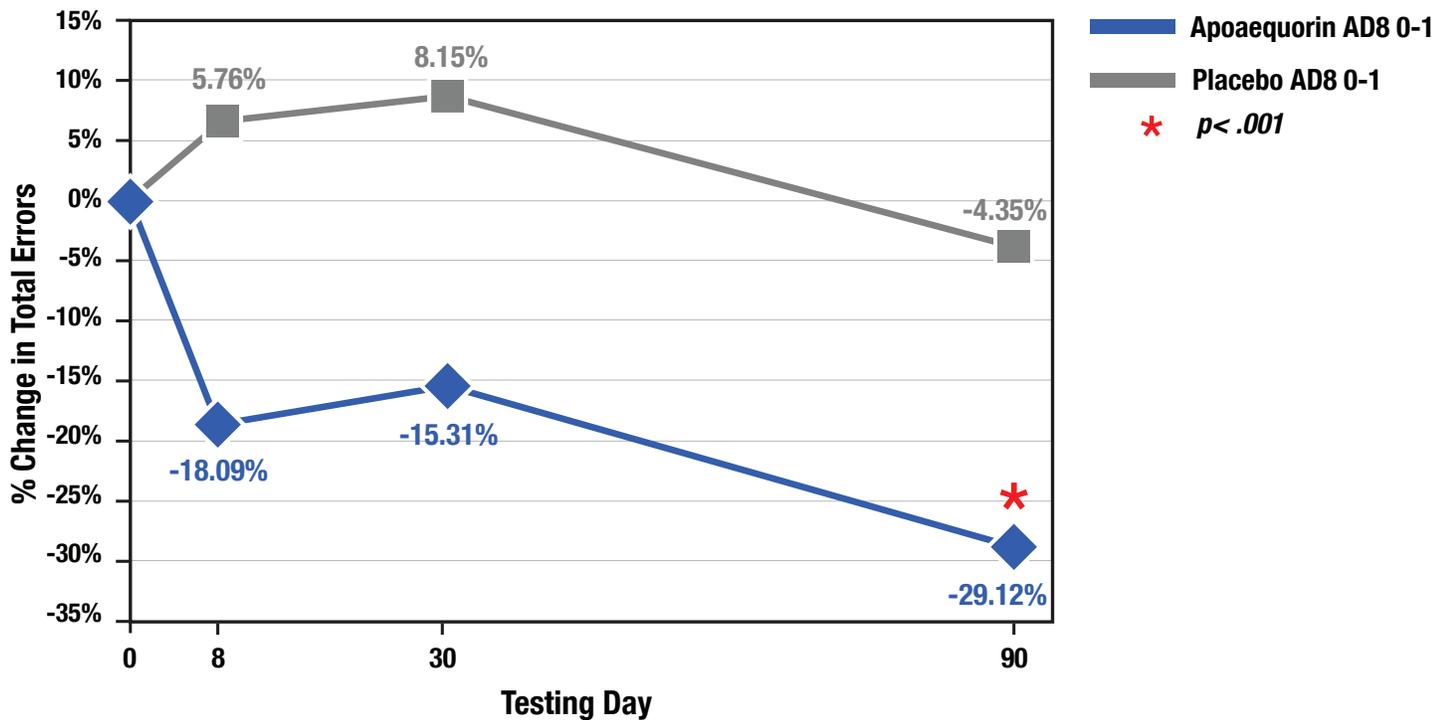


- Additionally, participants in the Apoaequorin arm within the cognitive range 0 to 1 on the AD8 showed significant change over time on the GML from Baseline/Day 0 to Day 90, ( $\mu= 12.81$ ,  $SD= 18.47$ ),  $t_{36}= 4.29$ ,  $p< .000$ , Cohen's  $d= 1.00$ . There was a decrease in total errors by 22.88% over the course of the study.
- 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%.

## Results

- 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 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 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**



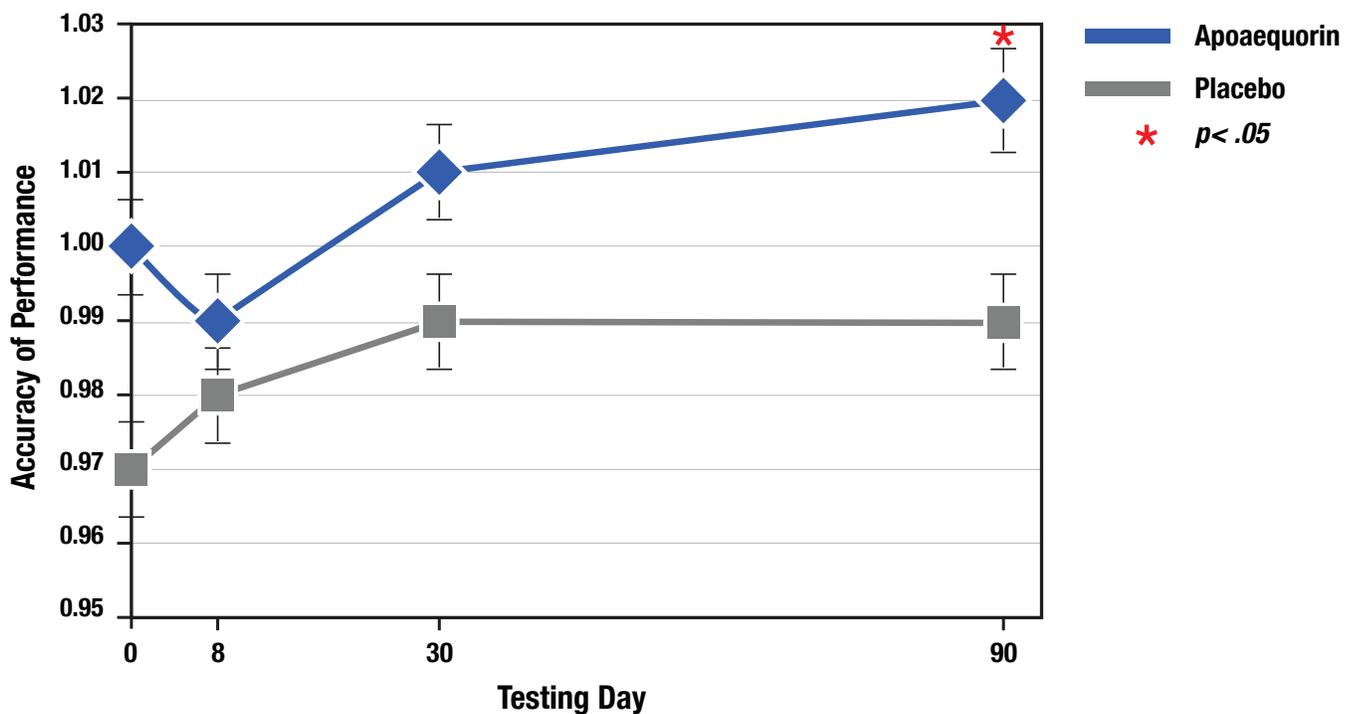
## Results

### 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**



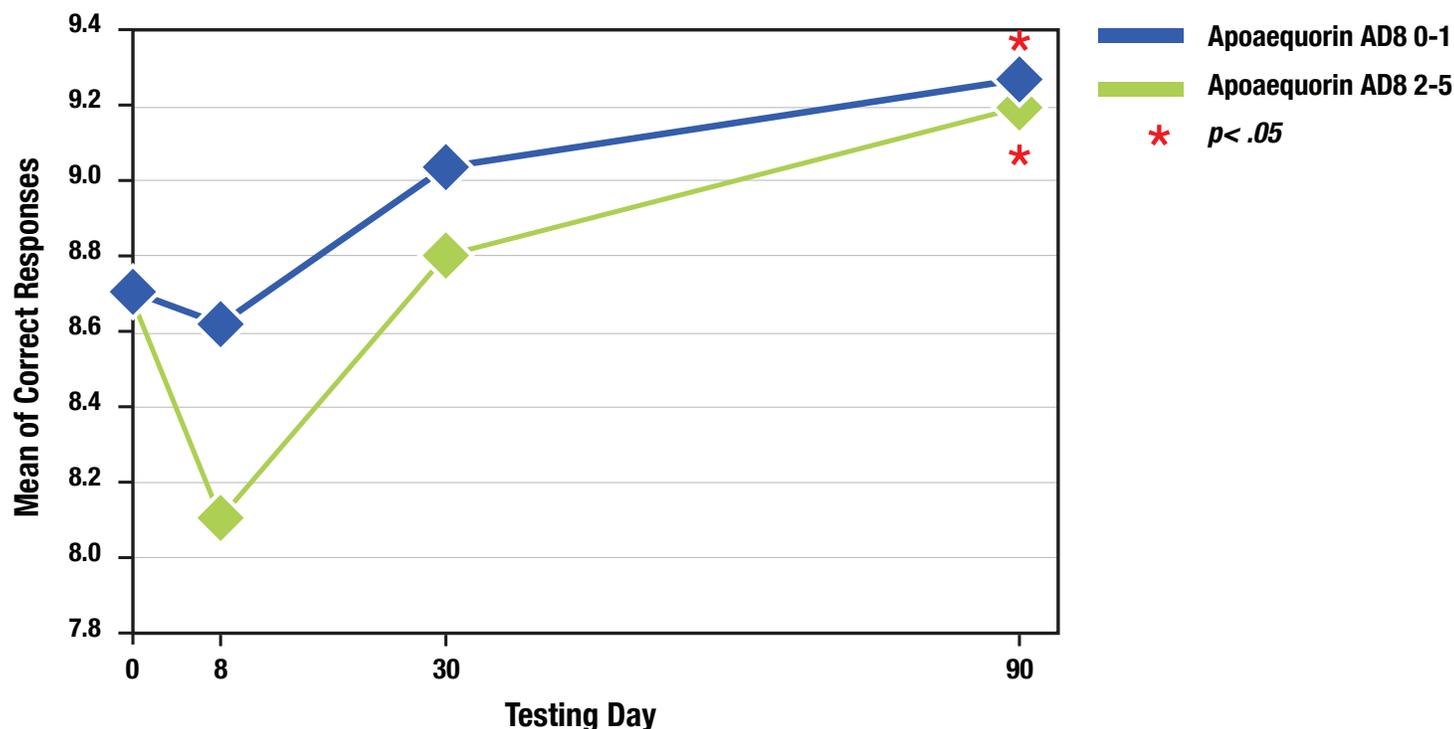
## Results

### 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 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 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

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Overall, participants in the Apoaequorin arm 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)

## Conclusion

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This study establishes a relationship between Apoaequorin and improvements on quantitative measures of cognitive function. Overall, the results suggest that Apoaequorin has a potential benefit in improving cognitive function associated with aging.

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