

Abstract Clinical Trial Synopsis QB-0011

QB-0011

TITLE:

Madison Memory Study: A Randomized, Double-Blinded, Placebo-Controlled Trial of Apoaequorin in Community-Dwelling Adults

SPONSOR:

Quincy Bioscience, LLC

PRINCIPAL INVESTIGATOR:

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OBJECTIVE:

The primary objective of the Madison Memory Study was to determine whether Prevacen® with apoaequorin (10 mg) improves quantitative measures of cognitive function in community dwelling adults

STUDY DATES:

3 December 2009 to 13 April 2011

REPORT DATE:

1 August 2016

INTRODUCTION

Apoaequorin is a protein originally found in a species of jellyfish (1). It is available commercially in a dietary supplement and has been determined to be safe (2) and non-allergenic (3).

Apoaequorin has been shown in laboratory studies to support neuronal cells (4), (5). Based on in vitro and in vivo animal studies (4), (5), (6), (7), we hypothesized that apoaequorin has the potential to enhance memory and cognitive function in humans. Previous work with apoaequorin in canines demonstrated cognitive enhancement (7).

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 adults. Community dwelling participants were randomized into either the Experimental group (apoaequorin) or Control group (placebo) at a ratio of 3:2. Participants in the Control group received capsules containing only white rice flour. Participants in the Experimental group 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.

To segregate participants by their level of self-reported cognitive impairment, a Baseline participant score

was acquired using the AD8 screening tool. The AD8 is a brief (8-question) screening tool that was developed to differentiate adults facing normal cognitive function from those with early signs of dementia (8), (9), (10). In this study, an AD8 score of 2 was used as a cut-off value to discriminate between those people who are cognitively normal or very mildly impaired (AD8 0-2) versus those with higher levels of impairment (AD8 3-8). Because Prevagen is a dietary supplement intended for healthy, non-demented individuals, results from the AD8 0-1 and AD8 0-2 subgroups are the most relevant to the efficacy of the product.

Quantitative, computerized cognitive tests were employed to examine the effect of apoaequorin over time and compared to placebo. The tests used in this study are part of the CogState Research battery and are adaptations of standard neuropsychological tests. CogState was selected for this study because its tests are brief, repeatable, and have shown little or no practice effects (11), (12), (13).

Nine CogState tests were used in this study: the International Shopping List (ISL), International Shopping List-Delayed Recall (ISRL), Groton Maze Learning (GML), Groton Maze Recall (GMR), Detection (DET), Identification (IDN), One Card Learning (OCL), One Back (ONB), and Two Back (TWOB) (Table 1).

Table 1 Cognitive Measurement Tests

Task	Cognitive Domain Measured
International Shopping List (ISL)	Verbal Learning
International Shopping List - Delayed Recall (ISRL)	Memory
Groton Maze Learning (GML)	Executive Function
Groton Maze Learning - Delayed Recall (GMR)	Memory
Detection (DET)	Psychomotor Function
Identification (IDN)	Attention
One Card Learning (OCL)	Visual Learning
One Back (ONB)	Working Memory
Two Back (TWOB)	Working Memory

Trained proctors administered the CogState tests. Participants completed testing sessions on five (5) occasions (Day(s) 0, 8, 30, 60, & 90). The primary efficacy variable was change from Day 0 (Baseline) to Day 90 on the CogState tests.

The ISL and the ISRL tests measure changes in verbal learning and working memory (13, 14). Verbal learning is the cognitive function associated with memorization and retention of a list of words. However, verbal learning is not solely the memorization of a list of words. It refers to the ability to learn information verbally (15). Verbal working memory is the ability to keep instructions in working memory and use them to perform a task. The ability to use verbal working memory is necessary to perform a task that is preceded by verbal instructions.

The ISL is a 12-word, three-trial, verbal list-learning test that is similar to other verbal list assessments. In the ISL, the presentation of stimuli and the recording of responses are facilitated by a trained proctor and recorded by the computer. Each 12-word list that is used is generated by the software and presented in a random order. The list is presented three (3) times to the participants. The ISL has good sensitivity to impaired/altered verbal memory. The ISRL is a repetition of the ISL list presented approximately 25 minutes after the initial three (3) trials. The ISRL measures verbal learning and delayed memory/recall.

The primary outcome measure for the ISL is the change in the total number of shopping list words participants are able to remember during three (3) iterations of the test. The primary outcome measure for the ISRL is the number of words recalled from the shopping list presented approximately 25 minutes earlier. For both tests, higher scores indicate better performance.

The GML and the GMR tests assess executive function and visual-spatial memory/problem solving (16). Executive function is comprised of high-level cognitive processes that help individuals complete complicated tasks and accomplish goals. Executive function refers to mental skills that are coordinated

in the frontal lobe and includes 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) (17).

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 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 is the total number of errors, with lower scores indicating better performance.

The DET test is a simple reaction time test that measures psychomotor speed. 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 test ends when 35 correct trials are recorded. Mean speed of performance for correct responses is the outcome measure. A lower score indicates better performance.

The IDN test is a choice reaction time test that measures visual attention. The participant must press the "Yes" key as quickly as possible when the presented card is red or "No" if it is black. The test ends when 30 correct trials are recorded. Mean speed of performance for correct responses is the outcome measure. A lower score indicates better performance.

The OCL test assesses visual attention and recognition memory. Participants are asked to respond "Yes" if the face-up card appeared previously in the test

and “No” if it did not. Six (6) cards were repeated in a total of 42 cards. Mean accuracy is the outcome measure. A higher score indicates better performance.

The ONB test assesses visual attention and working memory. Participants are asked to respond “Yes” if the face-up card is exactly the same as the card that immediately preceded it or “No” if it is not. The test ends when 30 correct trials are recorded. Mean speed of performance for correct responses is the outcome measure.

The TWOB test assesses visual attention and delayed recall. Participants are asked to respond “Yes” if the face-up card is exactly the same as the card that was shown two cards earlier. The test ends when 30 correct trials are recorded. Mean speed of performance for correct responses is the outcome measure.

MATERIAL

Participants in the Control group received capsules containing only white rice flour. Participants in the Experimental group received capsules containing apoaequorin (10 mg) and white rice flour.

STUDY SAMPLE

During the screening phase participants were interviewed about their medical history and physical activity. Eligibility criteria included the following: (1) healthy males and females not excluded by predetermined exclusion criteria; (2) age between 40 to 95 at Baseline (Day 0); (3) concerns related to memory issues; and (4) ability to comply with the study protocol and complete periodic computerized cognitive tests. Individuals were excluded if they had: (1) a history of uncontrolled hypertension; (2) untreated psychotic or major depressive disorder; (3) a significant neurological disease; or (4) the inability to adhere to the study protocol or complete periodic computerized cognitive tests.

A total of 218 participants, ages 40 to 91, with self-reported memory concerns were enrolled in the study. Two hundred and eleven (211) participants completed the study.

STATISTICAL ANALYSIS

The principal aim of the analysis was to compare the effects of apoaequorin (10 mg) versus placebo over time on the outcomes of the CogState Research tests. Data analyses were performed on the intention-to-treat population, which included all randomized subjects.

To assess whether sample selection bias occurred, unpaired t-tests (normal variables) or Wilcoxon ranked sum tests (skewed variables) were performed on the pre-treatment (Baseline) values for the Experimental and Control groups. Paired t-tests or Wilcoxon signed rank tests were also used to examine changes from Baseline to each follow-up visit. A mixed model repeated-measures analysis of covariance was employed to compare the treatment effects between the two groups. This methodology accommodated longitudinal data with repeated measures, the prevention of false positive associations due to interaction terms, and loss minimization of data due to missing observations. The model included the Experimental group, time, and the interaction term between the two (group x time). The Baseline value of each outcome variable was added to the model in light of the possible effect of Baseline differences on the results. Once a model was selected and fitted to the data for a particular outcome variable, the interrelationships between group, time, and Baseline were assessed.

The results were expressed as mean and standard error of the mean (SEM) with a value $p \leq 0.05$ (2-tailed) as a criterion for statistical significance. Statistical analyses were performed using SAS 9.3 software (SAS Institute, Cary, North Carolina).

Participants were segregated into analysis groups based on self-reported levels of cognitive impairment as measured by the AD8 screening tool. Because Prevagen is a dietary supplement intended for healthy, non-demented individuals, particular focus was placed on the AD8 0-1 and 0-2 groups, which included only those individuals with AD8 scores suggesting normal cognitive function or very mild impairment.

RESULTS

While no statistically significant results were observed over the entire study population, there were statistically significant results in the AD8 0-1 and AD8 0-2 subgroups. These subgroups contain individuals with either minimal or no cognitive impairment, and are the appropriate population for a dietary supplement intended for healthy, non-demented individuals.

Table 2 shows participants' characteristics and Baseline test outcomes. In the AD8 0-2 and AD8 0-1 subgroups, no statistically significant differences were noted in Baseline values between the Experimental and Control groups in any of the nine variables. The randomization was successful.

Table 2 Characteristics and Test Outcomes of the Participants at Baseline

	AD8 0-2			AD8 0-1		
	Placebo n=40	Apoaequorin n = 60	P Value	Placebo n = 24	Apoaequorin n = 37	P Value
Age	67.45 ± 10.30	64.23 ± 10.65	0.137	69.17 ± 8.391	64.78 ± 10.99	0.130
AD8	1.150 ± 0.802	1.017 ± 0.873	0.461	0.583 ± 0.504	0.405 ± 0.498	0.180
ISL	24.45 ± 4.075	25.01 ± 5.434	0.567	24.62 ± 3.499	24.48 ± 6.162	0.888
ISRL	8.275 ± 2.385	8.762 ± 2.336	0.336	8.208 ± 2.449	8.702 ± 2.654	0.469
GML	60.37 ± 21.08	58.59 ± 23.45	0.648	61.83 ± 21.54	57.64 ± 18.97	0.570
GMR	9.400 ± 5.424	8.898 ± 4.470	0.903	9.208 ± 4.211	9.324 ± 4.870	0.894
DET	2.500 ± 0.081	2.534 ± 0.104	0.089	2.503 ± 0.066	2.543 ± 0.095	0.077
IDN	2.726 ± 0.068	2.729 ± 0.072	0.913	2.733 ± 0.066	2.725 ± 0.069	0.624
OCL	1.005 ± 0.113	1.013 ± 0.107	0.583	1.016 ± 0.103	1.017 ± 0.103	0.972
ONB	1.298 ± 0.185	1.356 ± 0.163	0.188	1.313 ± 0.145	1.356 ± 0.156	0.298
TWOB	1.223 ± 0.164	1.251 ± 0.141	0.306	1.220 ± 0.168	1.244 ± 0.148	0.564

Notes on Table 2

1 All values are described with mean ± standard deviation (SD).

2 P value is based on unpaired t-test (normal variables) or a Wilcoxon ranked sum test (skewed variables).

Table 3 and 4 list the mean values and SD of outcomes on Day 0 and Day 90 for the AD8 0-1 AD8 0-2 subgroups, respectively. Within group p values and p values from the mixed model analysis are also reported.

Table 3 The Score Differences in the Two Groups Before and After Treatment (AD8 0-1)

Tasks	Placebo		Within p value	Apoaequorin		Within p value	Between Group P value			
	Day 0	Day 90		Day 0	Day 90		Group	Time	Group x Time	Base
ISL	24.62 ± 3.499	25.19 ± 5.163	0.373	24.48 ± 6.162	27.25 ± 5.106	0.002*	0.125	0.040*	0.279	<.0001*
ISRL	8.208 ± 2.449	8.904 ± 2.947	0.030*	8.702 ± 2.654	9.277 ± 2.614	0.091	0.704	0.134	0.897	<.0001*
GML	61.83 ± 21.54	51.00 ± 21.54	0.003*	57.64 ± 18.97	44.58 ± 13.69	<0.0001*	0.103	<.0001*	0.491	<.0001*
GMR	9.208 ± 4.211	8.809 ± 5.182	0.296	9.324 ± 4.870	6.444 ± 3.691	0.000*	0.011*	0.065	0.078	<.0001*
DET	2.503 ± 0.066	2.557 ± 0.096	0.005*	2.543 ± 0.095	2.530 ± 0.082	0.561	0.015*	0.146	0.021*	<.0001*
IDN	2.733 ± 0.066	2.727 ± 0.059	0.965	2.725 ± 0.069	2.723 ± 0.059	0.854	0.246	0.979	0.460	<.0001*
OCL	1.016 ± 0.103	1.018 ± 0.119	0.836	1.017 ± 0.103	1.049 ± 0.093	0.057	0.010*	0.330	0.193	<.0001*
ONB	1.313 ± 0.145	1.404 ± 0.160	0.015*	1.356 ± 0.156	1.397 ± 0.145	0.214	0.220	0.013*	0.388	<.0001*
TWOB	1.220 ± 0.168	1.321 ± 0.157	0.021	1.244 ± 0.148	1.312 ± 0.134	0.019*	0.747	0.004*	0.474	<.0001*

Notes on Table 3

1 Time is the number of visits since the initial Baseline visit and was coded as a categorical variable.

Table 4 The Score Differences in the Two Groups Before and After Treatment (AD8 0-2)

Tasks	Placebo		Within p value	Apoaequorin		Within p value	Between Group P value			
	Day 0	Day 90		Day 0	Day 90		Group	Time	Group x Time	Base
ISL	24.45 ± 4.075	25.50 ± 5.474	0.090	25.01 ± 5.434	27.68 ± 4.634	<0.0001*	0.324	0.000*	0.039*	<.0001*
ISRL	8.275 ± 2.385	9.000 ± 2.908	0.012*	8.762 ± 2.336	9.482 ± 2.400	0.002*	0.465	0.015*	0.703	<.0001*
GML	60.37 ± 21.08	50.02 ± 22.43	0.000*	58.59 ± 23.45	46.46 ± 18.78	<0.0001*	0.040*	<.0001*	0.463	<.0001*
GMR	9.400 ± 5.424	8.861 ± 5.938	0.229	8.898 ± 4.470	7.017 ± 4.722	0.001*	0.107	0.092	0.367	<.0001*
DET	2.500 ± 0.081	2.537 ± 0.099	0.045*	2.534 ± 0.104	2.533 ± 0.100	0.675	0.250	0.165	0.365	<.0001*
IDN	2.726 ± 0.068	2.732 ± 0.064	0.267	2.729 ± 0.072	2.725 ± 0.061	0.815	0.037*	0.780	0.108	<.0001*
OCL	1.005 ± 0.113	1.018 ± 0.121	0.292	1.013 ± 0.107	1.041 ± 0.100	0.046*	0.020*	0.437	0.357	<.0001*
ONB	1.298 ± 0.185	1.421 ± 0.156	<.0001*	1.356 ± 0.163	1.397 ± 0.140	0.081	0.944	0.000*	0.223	<.0001*
TWOB	1.223 ± 0.164	1.317 ± 0.176	0.002*	1.251 ± 0.114	1.302 ± 0.127	0.028*	0.934	0.000*	0.290	<.0001*

Notes on Table 4

1 Time is the number of visits since the initial Baseline visit and was coded as a categorical variable.

International Shopping List and International Shopping List-Delayed Recall

Figure 1 shows the average percentage change in ISL scores from Baseline to each visit in participants with AD8 scores of 0-1. The Experimental group demonstrated an 11.29% increase in the number of correct responses, while the Control group exhibited a 2.30% improvement. As compared to Baseline, a statistically significant difference was observed in the Experimental group ($p=0.002$), but not the Control group ($p=0.373$). A trend towards significance was shown in comparing the Experimental group's results to the Control group's results ($p=0.125$).

Figure 2 shows the average percentage change in ISL scores from Baseline to each visit in participants with AD8 scores of 0-2. The Experimental group showed a 10.68% increase in the number of correct responses, while the Control group showed a 4.29% increase. Compared to Baseline, the number of correct responses was significantly increased in the Experimental group ($p<0.0001$), but not in the Control group ($p=0.090$). The two groups tended to show a group difference of greater magnitude. Nonetheless, a significant difference between the Experimental and Control groups was not observed ($p=0.324$). This may be the result of a score reduction that occurred at visit 2 in the Experimental group.

As compared to Baseline, both groups showed a statistically significant or nearly significant increase in ISRL scores in participants with AD8 scores of 0-2 and participants with AD8 scores of 0-1. Significant differences between the Experimental and Control groups were not observed.

Figure 1: Percentage Change of ISL (AD8 0-1)

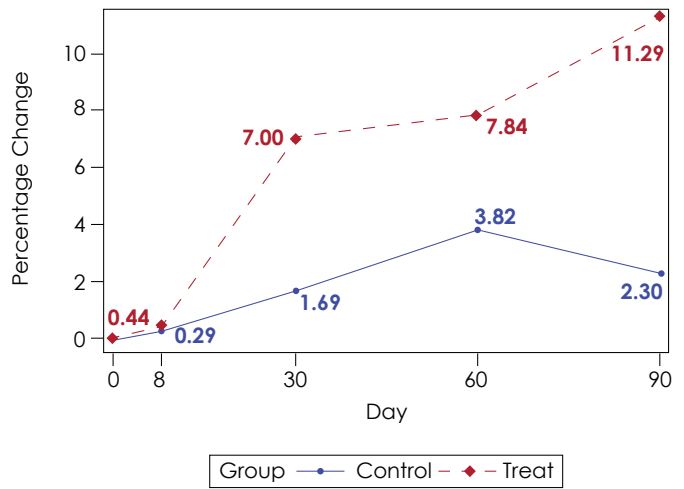
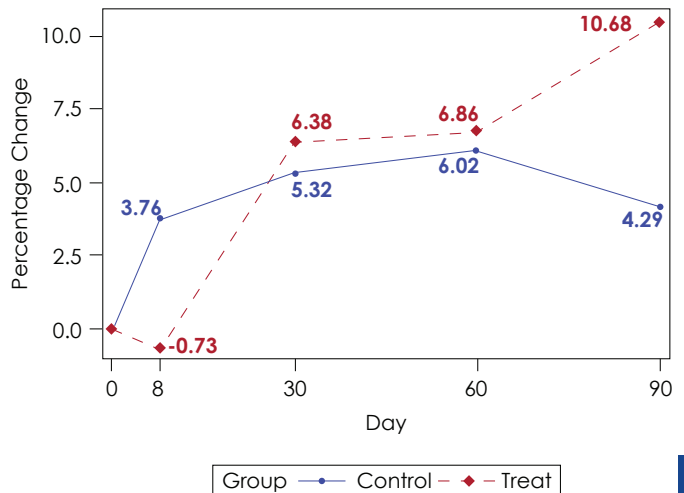


Figure 2: Percentage Change of ISL (AD8 0-2)



Groton Maze Learning

Figure 3 shows the average percentage change in GML scores from Baseline to each visit in participants with AD8 scores of 0-1. As compared to Baseline, both groups showed statistically significant reductions in total errors (Experimental $p < 0.0001$, Control $p = 0.003$). There was a trend towards significance in comparing the results from the Experimental group to the Control group ($p = 0.103$).

Figure 4 shows the average percentage change in GML scores from Baseline to each visit in participants with AD8 scores of 0-2. The Experimental group demonstrated a 20.70% reduction in the number of moves required to traverse a 10x10 maze, while the Control group exhibited a 17.14% reduction. As compared to Baseline, both groups experienced a statistically significant reduction in the number of moves required (Experimental $p < 0.0001$, Control $p = 0.0002$). The Experimental group's results were statistically significant as compared to the Control group ($p = 0.0400$).

Groton Maze Recall

Figure 5 shows the average percentage change in GMR scores from Baseline to each visit in participants with AD8 scores of 0-1. The Experimental group required 30.89% fewer moves to complete the maze between Days 0 and 90. The Control group experienced a 4.33% reduction. Compared to Baseline, a statistically significant change was observed in the Experimental group ($p = 0.000$), but not the Control group ($p = 0.296$). The Experimental group's results were statistically significant compared to the Control group ($p = 0.011$).

Figure 3: Percentage Change of GML (AD8 0-1)

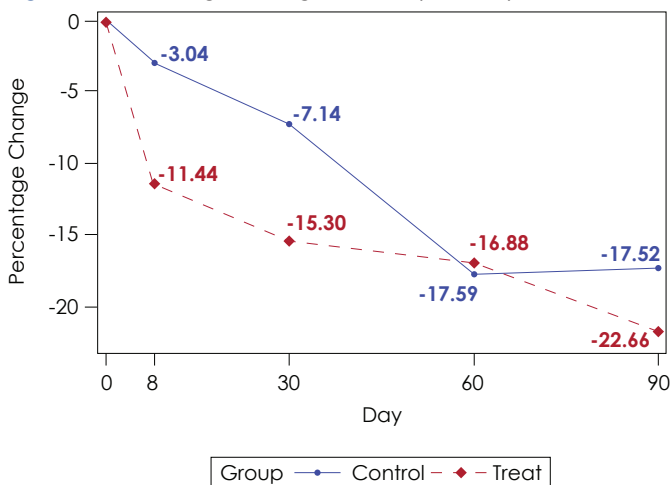


Figure 4: Percentage Change of GML (AD8 0-2)

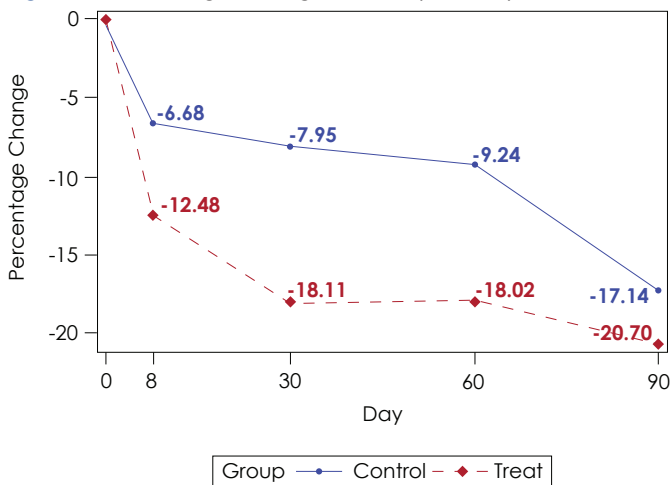


Figure 5: Percentage Change of GMR (AD8 0-1)

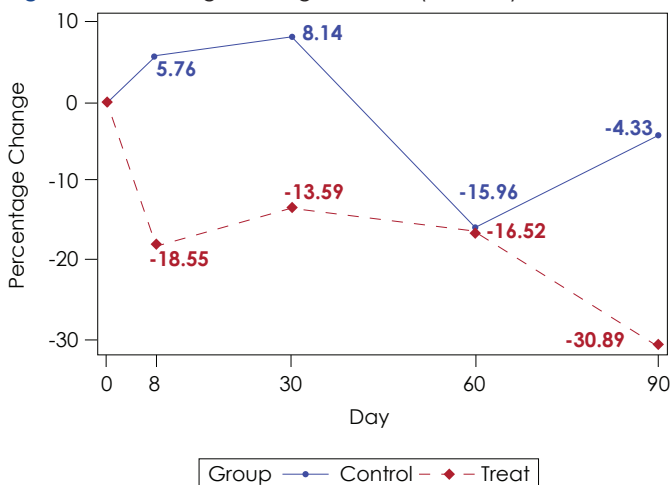


Figure 6 shows the average percentage change in GMR scores from Baseline to each visit in participants with AD8 scores of 0-2. The total number of moves required to traverse the maze in the Experimental group decreased by 21.14% between Baseline and Day 90, compared to only a 5.73% decrease in the Control group. As compared to Baseline, a statistically significant difference was shown in the Experimental group ($p = 0.001$), but not the Control group ($p = 0.229$). The Control group showed an initial decrease of magnitude related to Baseline value followed by a regain in the third visit. There was a trend toward significance in the total number of errors in the Experimental group as compared to the Control group ($p = 0.107$).

Detection and Identification

Figure 7 shows the average percentage change in DET scores from Baseline to each visit in participants with AD8 scores of 0-1. A statistically significant difference was shown in the Experimental group as compared to the Control group ($p = 0.015$). For the participants with AD8 scores of 0-2, the Experimental group outperformed the Control group at all post intervention visits, but did not reach the significance level ($p = 0.250$).

Figure 8 shows the average percentage change in IDN scores from Baseline to each visit in participants with AD8 scores of 0-2. The IDN results showed a statistically significant difference between the two groups ($p = 0.037$). For participants with AD8 scores of 0-1, the between group differences were not statistically significant ($p = 0.246$).

Figure 6: Percentage Change of GMR (AD8 0-2)

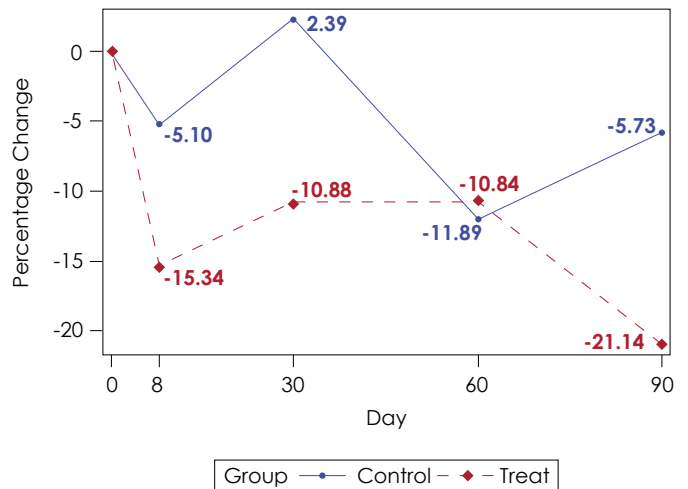


Figure 7: Percentage Change of DET (AD8 0-1)

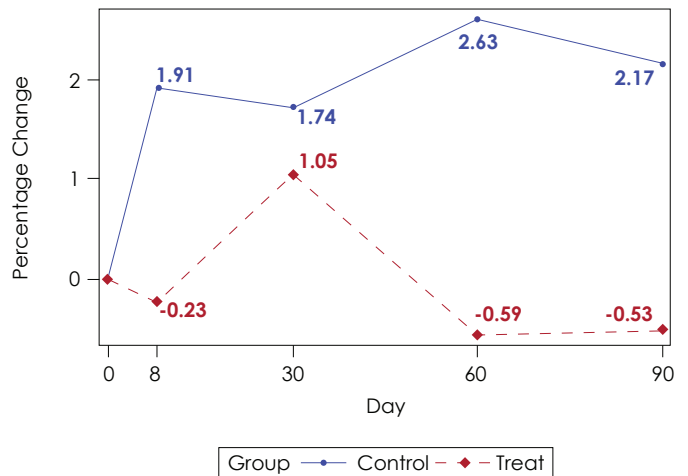
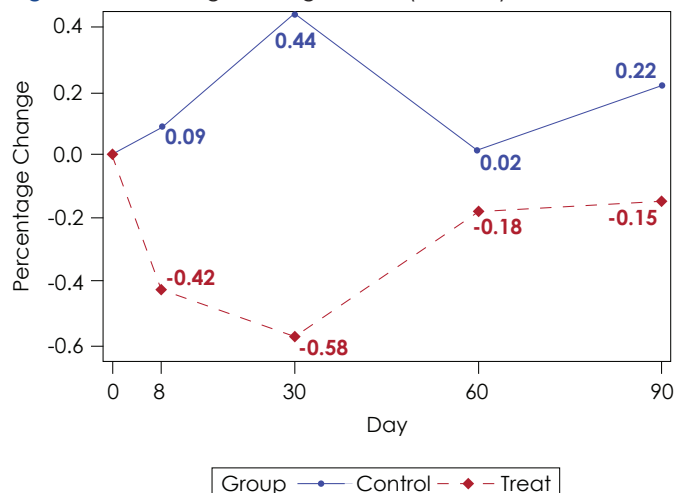


Figure 8: Percentage Change of IDN (AD8 0-2)



One Card Learning

Figure 9 shows the average percentage change in OCL scores from Baseline to each visit in participants with AD8 scores of 0-1. The Experimental group experienced a 3.164% change as compared to a 0.245% change in the Control group. Compared to Baseline, a nearly statistically significant difference was observed in the Experimental group ($p=0.057$), but not the Control group ($p=0.836$). The difference between the two groups was statistically significant ($p=0.010$).

Figure 10 shows the average percentage change in OCL scores from Baseline to each visit in participants with AD8 scores of 0-2. Compared to Baseline, a significant difference was seen in the Experimental group ($p=0.046$), but not the Control group ($p=0.292$). A statistically significant difference was observed between the groups ($p=0.020$).

Figure 9: Percentage Change of OCL (AD8 0-1)

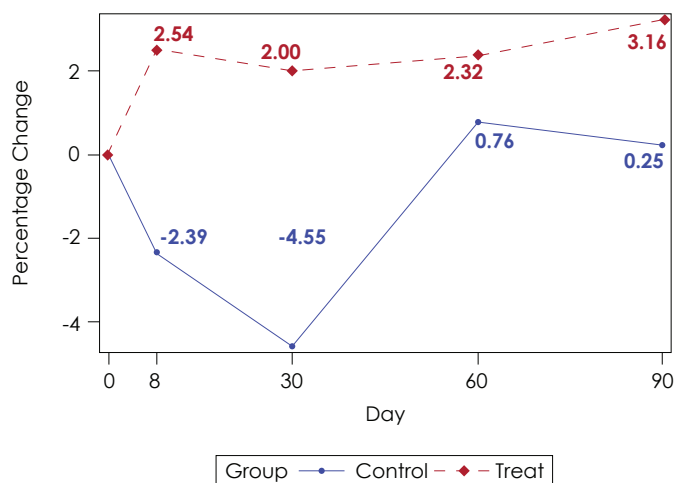
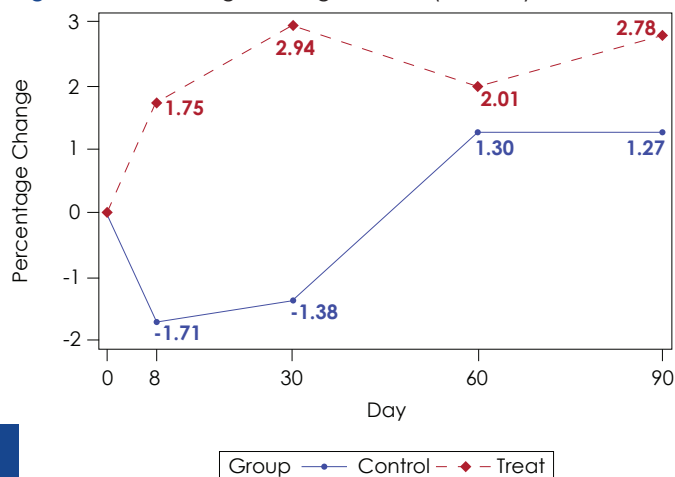


Figure 10: Percentage Change of OCL (AD8 0-2)



One Back and Two Back

In participants with AD8 scores of 0-1 and 0-2, significant differences between the Experimental and Control groups were not observed on either the One Back or the Two Back tests.

Adverse Events

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

Discussion

This study was designed to examine the effect of Prevagen® on cognitive function in a study population of community dwelling adults with self-reported cognitive difficulties or concerns. Changes in cognitive function were quantitatively assessed using tests from the CogState Research battery.

Participants in the Experimental group with AD8 scores of 0-1 experienced statistically significant improvements, as compared to the Control group, on the following tests: GMR ($p=0.011$), DET ($p=0.015$), and OCL ($p=0.010$). These participants also experienced trends toward statistical significance on the GML and ISL tests ($p=0.103$, $p=0.125$). Participants in the Experimental group with AD8 scores of 0-2 experienced statistically significant improvements, as compared to Control group participants, on the following tests: GML ($p=0.040$), IDN ($p=0.037$), and OCL ($p=0.02$). These participants also experienced a trend toward significance on the GMR test ($p=0.107$). These data support the hypothesis that oral supplementation with Prevagen supports cognitive function in healthy, non-demented individuals.

Conclusion

Prevagen demonstrated the ability to improve aspects of cognitive function in participants with either normal cognitive function or very mild impairment, as determined by AD8 screening.

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