A Double-Blind, Placebo-Controlled Trial of Acetyl-L-Carnitine and Alpha-Lipoic Acid for Age-Associated Memory Impairment

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ABSTRACT

BACKGROUND: Acetyl-L-carnitine and alpha-lipoic acid ingested together (ALC+ALA) results in memory and other improvements in rats (1) and dogs (2). This double-blind, parallel group study was designed to determine if this combination improves age-associated memory impairment (AAMI).

METHODS: 120 AAMI individuals were randomly assigned placebo, low or high dose ALC+ALA (ALC 1000+ALA 400 or ALC 2000+ALA 800 mg/day; Juvenon, Inc.) for three months. The Rey Auditory Verbal Learning Test was administered by telephone twice at baseline and on days 28, 56 and 84.

RESULTS: Working (immediate) memory improved, but no significant change was observed in delayed memory. The ALC+ALA combination was not associated with side effects as would be expected from their history as dietary supplements. To increase statistical power in the current study, training and baseline scores were averaged and participant data sets with signal-to-noise ratios below 10 were excluded (final N=63). In this study, working memory improved significantly in the high-dose intervention group compared to within-group baseline (p=0.01) and to placebo (p=0.048). A clear dose-response effect was evident: placebo scores improved by 0.9% while low and high dose intervention groups improved by 9.3% and 17.6% with most improvement occurring by day 56 (p=0.053). Noise exclusion reduced the high-dose (day-84) vs. placebo p-value from 0.190 to 0.048 and increased percent improvement from 16.2% to 17.6%. Future improvement in study design could increase the power of the study to detect effects.

CONCLUSIONS: A daily dose of ALC+ALA enhanced working memory in AAMI individuals. Statistical significance was only achieved after baseline averaging and exclusion of low signal-to-noise data. Because multiple tests of significance were applied, these results must be confirmed by follow-up investigations before a final conclusion is reached.

BACKGROUND:

Thousands of studies in humans and animals have shown that learning and memory decline progressively after the organism reaches maturity and passes into later stages of its lifespan (1). The magnitude of decline in humans depends upon the demands of the test administered. For example, among the memory subtests of the Wechsler Adult Intelligence Scale, the decline expected between age 25 and 75 ranges from negligible, for recalling vocabulary definitions, to over 70% for recalling abstract geometric associations quickly (2). On other tests related to critical tasks of daily life, such as recalling the names of persons to whom one is introduced, the decline over the same 50 year span exceeds 50%, even when older subjects with common medical problems that can impair memory are excluded from the comparison (3). Declines of a similar magnitude have been reported on numerous tasks that are critical in daily life (4, 5). Thus, although memory loss in later life may be "normal" it may also be highly problematic.

In order to facilitate research into the nature and, particularly, the improvement of memory loss among mature adults, the National Institute of Mental Health (NIMH) convened a consensus conference in 1985 that developed the diagnostic construct Age-Associated Memory Impairment (AAMI; 7). The term describes healthy persons over 50 years of age who complain they have experienced gradual memory loss since early adulthood and who show evidence on standard neuropsychological tasks that such loss has occurred. Specific inclusion and exclusion criteria were provided to assure that memory loss is not the result of medical or psychiatric disorders and that it is not so severe that the individual could be diagnosed as suffering from dementia. In more recent years, both the American Psychiatric Association (8) and the American Psychological Association (9) have adopted identical diagnostic constructs, although modifying the term somewhat to Age-Related Cognitive Decline (ARCD). The construct is described in the fourth edition of the American Psychiatric Association's Diagnostic and Statistical Manual (DSM-IV;8) not as a psychiatric disorder, but as a developmental condition that warrants attention to improvement.

During the past two decades, multiple neurochemical systems have been implicated in AAMI (e.g. 10-11) and multi-center clinical trials have been conducted (e.g. 13). Several compounds have been found to have positive effects in AAMI, but with one exception (phosphatidylserine), side-effect profiles were such that they were considered unacceptable in AAMI. For example, side effects associated with cholinesterase inhibitors that have not blocked regulatory approval for treating Alzheimer's disease (AD) would probably be unacceptable for treating the more modest cognitive symptoms characteristic of AAMI.

Acetyl-L-Carnitine (ALC) has not been studied in AAMI, to our knowledge, but there is an extensive, if somewhat inconsistent, literature gathered over many years (e.g. 14-16) suggesting that the compound is safe and may improve cognition in elderly patients suffering from Alzheimer's disease (AD) and other disorders. Inconsistent findings likely result from the different populations in which ALC has been studied, as well as widely differing dosages and durations of using the supplement. AAMI may be a more appropriate target for ALC than Alzheimer's disease (AD) or other indications in which the compound has been studied because the same neurochemical deficits underlie both conditions but there is generally less neuronal degeneration in AAMI and the framework of cognition remains largely intact. Because AAMI subjects are capable of responding to complex neuropsychological tests, much more subtle drug effects can be detected.

METHODS

Study subjects

More than one thousand (i.e., 1326) individuals were interviewed for study admission, 236 were screened for the study and 120 were selected and randomly assigned one of the two dosage levels of the investigational compound or placebo. Subject interviews and selection were conducted via telephone; subjects were not seen in the clinic. A medical monitor was available to judge whether medical exclusion criteria were met, as well as to assess the severity of any adverse events, and decide about subject withdrawal should an adverse event occur. The study was promoted in the media and potential study subjects contacted study coordinators by telephone or email. Subjects were blind to treatment group assignment and were offered a three month supply of the investigational product at the end of the 12 week study. Entrance criteria were as follows:

Inclusion Criteria

Subjects eligible for inclusion were men or women between 50 and 79 years of age who met the following criteria for AAMI: (I) Complaints of memory loss in everyday life as reflected by a score of 25 or more on the Memory Complaint Questionnaire (MAC-Q; 29). (II) Memory test performance at least one standard deviation below the mean established for young adults but within the range of normality for their age on the Logical Memory I or the Verbal Paired Associates 1 subtest of the Wechsler Memory Scale Revised (WMS-R;30). The tests were administered by telephone and the acceptable range of scores was 11 to 19 inclusive for Logical Memory and 0 to6 for Verbal Associates (Hard Items). (III) Willingness to comply with study procedures and provide informed consent.

Exclusion Criteria

Subjects who meet any of the following criteria were deemed ineligible for inclusion in the study. (I) Clinically significant cardiovascular, hepatic, renal, endocrine, neurologic, or psychiatric disorders that could be responsible for memory loss in the judgment of the medical monitor. Subjects with a history of diabetes mellitus, symptoms of hypoglycemia (sweating, intense hunger, trembling, weakness, palpitations, or difficulty speaking), seizure disorder (excluding febrile seizures), lactose intolerance, or reported history of a head injury that resulted in unconsciousness for greater than 20 minutes. (II) Subjects who did not have health insurance. (III) Depression as determined by a score of 11 or higher on the Geriatric Depression Scale (GDS;31). (IV) Use of any of the following drugs or dietary supplements within 30 days preceding baseline evaluation or reasonable expectation that such drugs would be required during the course of the study: Antipsychotic agents, benzodiazepines, prescription sleeping aids (short acting prescription sleeping aids such as Ambien, are allowed, but may not be taken within 8 hours prior to testing); Benadryl or other sedating antihistamines are not allowed within 8 hours of testing; cholinesterase inhibitors; Central Nervous System (CNS) stimulants, e.g. amphetamine, thylphenidate, modafinil; dietary supplements containing Huperzine A, Ginkgo biloba, phosphatidylserine, omega-3 fatty acids, or any product containing ALC or ALA; any investigational drug; women who were pregnant or breast-feeding;
women of child-bearing potential not using a medically acceptable method of birth control [hormonal (oral or transdermal), or double-barrier method]; subjects who had taken or were currently taking the Juvenon Cellular Health Supplement.

Demographic Characteristics

		Placebo	Low Dose	High Dose	Overall
Age	N	41	39	40	120
	Mean (SD)	61.7 (8.58)	58.9 (7.0)	61.5 (7.11)	60.7 (7.65)
	Median	62	57	61	60
	Min, Max	50,79	50,74	50,79	50,79
Sex (n, %)	Male	26 (63.4)	25 (64.1)	26 (65.0)	77 (64.2)
	Female	15 (36.6)	14 (35.9)	14 (35.0)	43 (35.8)
Ethnicity	White	38 (92.7)	35 (89.7)	34 (85.0)	107 (89.2)
	Black	0 (0.0)	1 (2.6)	1 (2.5)	2 (1.7)
	Asian	1 (2.4)	0 (0.0)	0 (0.0)	1 (0.8)
	Hispanic	1 (2.4)	0 (0.0)	2 (5.0)	3 (2.5)
	American Indian	0 (0.0)	2 (5.3)	2 (5.0)	4 (3.4)
	Other	1 (2.4)	1 (2.6)	1 (2.5)	3 (2.5)

Design

This was a fully randomized, double-blind, placebo-controlled, parallel-groups study of the cognitive effects of two doses of an ALA/ALC combination among subjects with AAMI. These compounds are dietary supplements currently sold in the United States.

Testing was conducted by telephone. Volunteers phoned study coordinators or visited a website where study procedures were explained. Inclusion and exclusion criteria were assessed by telephone and Informed Consent documents were explained by phone and
mailed or emailed to each prospective subject. Subjects were administered both the Geriatric Depression Scale and the WMS-R subtests by phone to determine eligibility. This information was then reviewed by the investigators and a decision made in each case as to eligibility for the study. Subjects found eligible were contacted by email or telephone to insure that they understood study procedures and were motivated to comply.

Subjects who passed this final screen were enrolled and instructed that they would receive a one month supply of tablets by express mail. Study subjects were then administered the Rey Auditory Verbal Learning Test, The MAC-S and the GDS to establish baseline values. This process was repeated the following day and then 28 days after baseline, 56 days post-baseline, and 84 days after baseline a final test session was conducted. Upon completion of the study, subjects were asked to give their opinion as to whether the study compounds improved memory or were associated with any side effects. During the entire study, subjects were reminded weekly by email or telephone to take the tablets. Also, subjects were contacted by email or phone the week prior and the day prior to all scheduled test days. Each subject was asked to count the remaining pills on days 28, 56 and 84. Subjects not taking between 80-100% of the study compound on days 28 or 56 were counseled on the need to carefully comply with the protocol. Subjects received telephone contacts (days 2, 14, 42, and 70) from the study staff to improve dosing compliance.

Outcome Measures

The Rey Auditory Verbal Learning Test *(RAVLT;17)*. In this test a list of 15 words is read to the subject five times and recall is assessed after each reading. After the fifth trial another list of "interfering" words is read and subjects are asked to recall those words. Subjects were then asked to recall the original list. Again, 30 minutes later in a "delayed recall" trial they were asked to recall the original list. The RAVLT was administered between one and two hours after study compound administration. Every attempt was made to complete the test between 6:00 am and 1:00 pm.

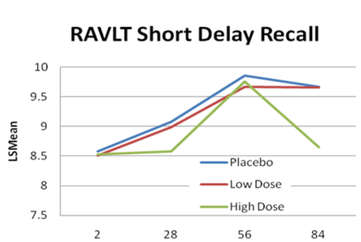
Memory Assessment Clinics Self-Rating Scale (MAC-S;18)
This is a self-rating scale that includes 10 "ability to remember" items and 2 global rating items. These latter items assess overall comparison to others and comparison to the best one's memory has ever been.

Statistics

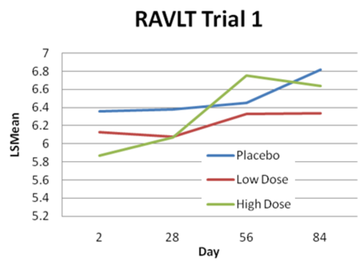
Change from baseline to the final evaluation (Day84) was analyzed on each outcome measure derived from the RAVLT and MAC-S using analysis of covariance procedures, with the baseline (Study Day 1) score as the covariate. The primary outcome measures were the two delayed recall trials on the RAVLT. Missing data were not imputed and only subjects who were compliant at day 84 were included in the primary analysis. Analyses of all other outcome variables were conducted in the same manner. During secondary reanalysis, all records that were complete though day 56 were examined to determine if the signal to noise ratio (S/N) of the first two RAVLT scores (screening and baseline) was equal to or greater than ten. S/N is defined as the reciprocal of the relative percent standard deviation. Data sets for which the S/N was below ten were discarded. Remaining scores were then subjected to Kolmogov-Smirnov tests to determine if distributions were normal for both the primary endpoint (baseline to final RAVLT scores) and for change in working memory, the first score during each RAVLT measurement). Student's t-tests were then applied to determine if placebo and high-dose intervention scores for the primary endpoint and also working memory were different at the 95% confidence level.

RESULTS

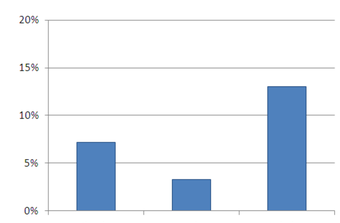
RAVLT scores displayed relatively high session-to-session standard deviation as if the statistical power of the study was marginal. Long delay recall results (not shown) followed a similar pattern: average scores for all groups were surprisingly high on day 56 and then decreased on day 84, raising the possibility of seasonal interference of some kind. For both short and long delayed recall, the placebo and high dose scores decreased most between days 56 and 84.



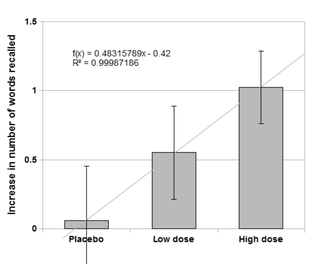
Working memory (RAVLT trial 1) scores also were relatively unstable across sessions, however high dose intervention group scores improved most and remained relatively high.



Replotting the same data showed relative improvement for placebo and treatment groups more clearly.



The data set was then cleaned by removing low signal to noise results (with high within-person standard deviations). Variability was further reduced by averaging screening and baseline scores. A clear dose response effect then became evident and the p value for high dose vs. baseline scores decreased to 0.048.



DISCUSSION

The cleaned data set was further examined by increasing and decreasing the S/N threshold to determine if the dose response curve at S/N >= 10 was simply a matter of chance. Effect size rose as high as 22.4% and a dose response curve was present from S/N thesholds between 5 and 15 when either day 56 or 84 scores were included, suggesting that working memory improvement in the high dose intervention group may be real rather than a chance effect.

The combination of acetyl-L-carnitine and alpha-lipoic acid has been studied for over ten years and has been discussed in at least 42 publications. During this time most studies have reported positive, beneficial results however others have been negative (e.g. 19). To our knowledge none of the negative studies has included a formal power analysis to estimate the likelihood that failure to replicate earlier positive studies resulted simply from inadequate statistical power. The present positive result, seen only after removal of the "noisiest" data sets, indicates that statistical power and the signal to noise ratio are critical and can determine whether or not the outcome of a study is positive at the 95% confidence level.

CONCLUSIONS

By applying the statistical methods described in this report the results obtained showed a clear effect of the two nutrients on working memory in humans with AAMI.

Acetyl-L-carnitine and alpha-lipoic acid have been found, together and separately in a variety of model systems and human trials, to have positive effects on a number of biological functions. Positive effects include improved mitochondrial function, cognitive performance, hearing, blood pressure, immunity, stroke resistance, reduced adverse effects from chemotherapy, and decreased neurodegeneration associated with Parkinson's and Alzheimer's. These results and those reported in this study warrant larger scale clinical trials to further evaluate potential benefits for humans.

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