

Evaluation of Memory Span in Normal Healthy Volunteers Following Six Weeks Dietary Supplementation with a New Functional Drink.

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Abstract

A new functional drink containing Iodine in the form seaweed extract and supplemented with amino acids was tested in normal healthy volunteers of age 50 to 75 years. A standard memory span test was used to access 147 participants over a six week period. A statistically significant increase in memory was seen after the six week program ($p < 0.005$).

Introduction

Reduction of cognitive function is a common effect of ageing. The decline can begin as early as 45 years of age (ref 1). In some the decline becomes sufficiently severe to describe the condition as a disease state e.g. Alzheimer's disease or dementia.

While substantial research is ongoing to treat these disease states little is done to address the problem of reducing cognitive function in 'normal' healthy individuals. A new drink has been developed for incorporation into the daily diet with a view to boosting cognitive function. The product contains a number of ingredients to help maintain healthy function. Seaweed extract is a rich source of nutrients and in particular Iodine (ref 2 and 3) which already has a European Food Safety Authority approved health claim for improved cognitive function. The formulation included are L-Arginine HCl and L-Citrulline Maleate to promote Nitric Oxide production in the endothelium of blood vessels thus improving circulation (ref 4, 5, 6). Also included is Acetyl-L-Carnithine which can cross the blood brain barrier and promote better energy utilisation in the brain. It is reported in double-blind controlled studies to have beneficial effects in major depressive disorders and Alzheimer's disease (AD), both of which are highly prevalent in the geriatric population. (ref 7).

By virtue of the Iodine content of seaweed extract, this product can include a health claim for maintaining cognitive function approved by the European Food Safety Authority (EFSA). (Ref 8). The aim of this study was to confirm the EFSA health claim and to quantify the effect.

Materials and Methods

The study was executed by an independent research company 'Backcheck'. Slainte Eol Teo (Five Sciences) provided the product.

This was a multi-centre study in normal healthy volunteers between the age of 50 and 75. Participants agreed to drink a 250ml bottle of the test product for a period of six weeks. They completed a product evaluation questionnaire and agreed to undergo a memory test every two weeks. This involved a memory test at time 0 (T0) before the commencement of the study and tests at two weeks (T2), four weeks (T4) and six weeks (T6). Exclusion criteria were age, pre-existing health conditions and allergies to any of the ingredients.

In this study a standard memory span test was used. In psychology and neuroscience, **memory span** is the longest list of items that a person can repeat back in correct order immediately after presentation (ref 9, 10 and 11). Items may include words, numbers, or letters. In this case random letter strings were used. Subjects were presented with a series of strings of letters of increasing

complexity (2, 4, 6, 8, 10 and 12 random letters) and immediately asked to recall the string. At each time point (0, 2 weeks, 4 weeks and 6 weeks) a new set of tests were used to avoid learning effects. Results were collected for ability to recall the string in the correct sequence (sequential) or to recall the string but not in the correct sequence (non-sequential). Memory span is a common measure of short-term memory. It is also a component of cognitive ability tests such as the Wechsler Adult Intelligence Scale (WAIS). Backward memory span is a more challenging variation which involves recalling items in reverse order. This report analyses the results of the memory tests.

Results

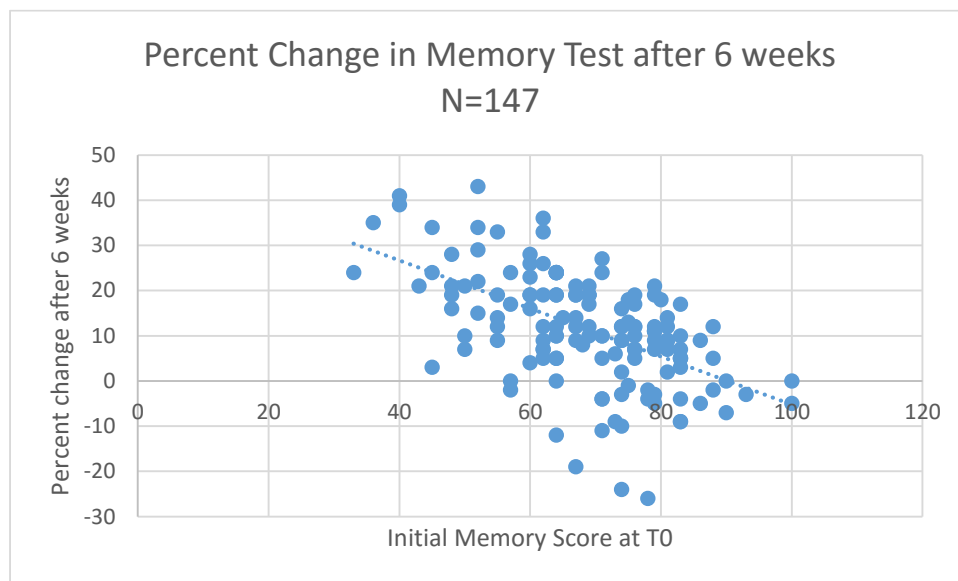
Efficacy Evaluation and Statistics

147 Normal volunteers between the ages of 50 and 75 completed the 6 week study. 121 (82%) showed an improvement in cognitive function when their initial memory test was compared to the memory test after 6 weeks taking the drink. Memory tests were analysed in two ways on the one hand where the subject recalled the full stream of letters in sequence, or alternatively, could remember all but in the wrong sequence. Including sequence increases the difficulty of the test

Figures 1 and 3 shows the memory test results for all 147 subjects (sequential and non-sequential).

Figure 1

Sequential



The results were stratified depending on the initial test scores. Subjects scoring 100% on the first test had no potential for memory improvement. Figures 2 and 4 shows the tiered nature of the response to the product (sequential and non-sequential).

Figure 2

Sequential

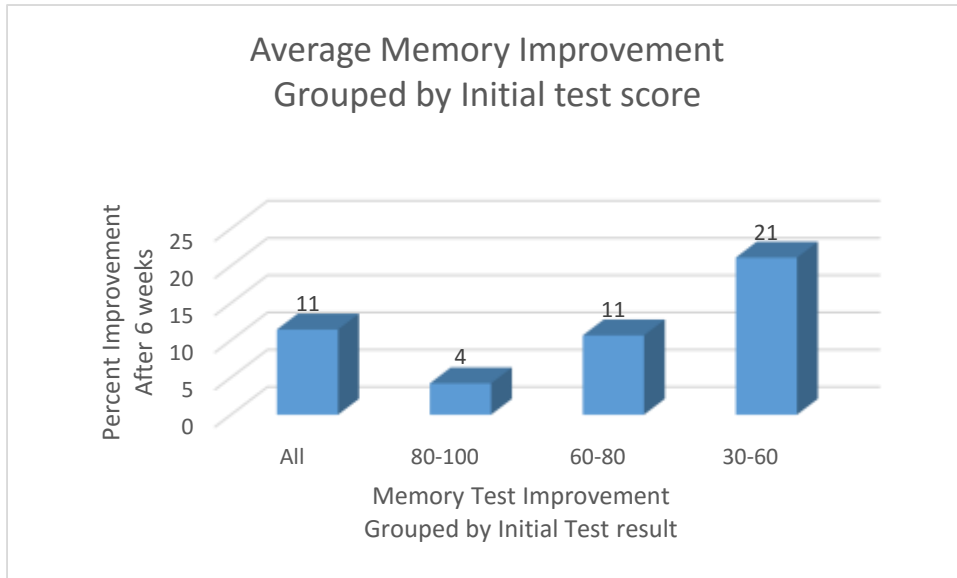


Figure 3

Non-Sequential

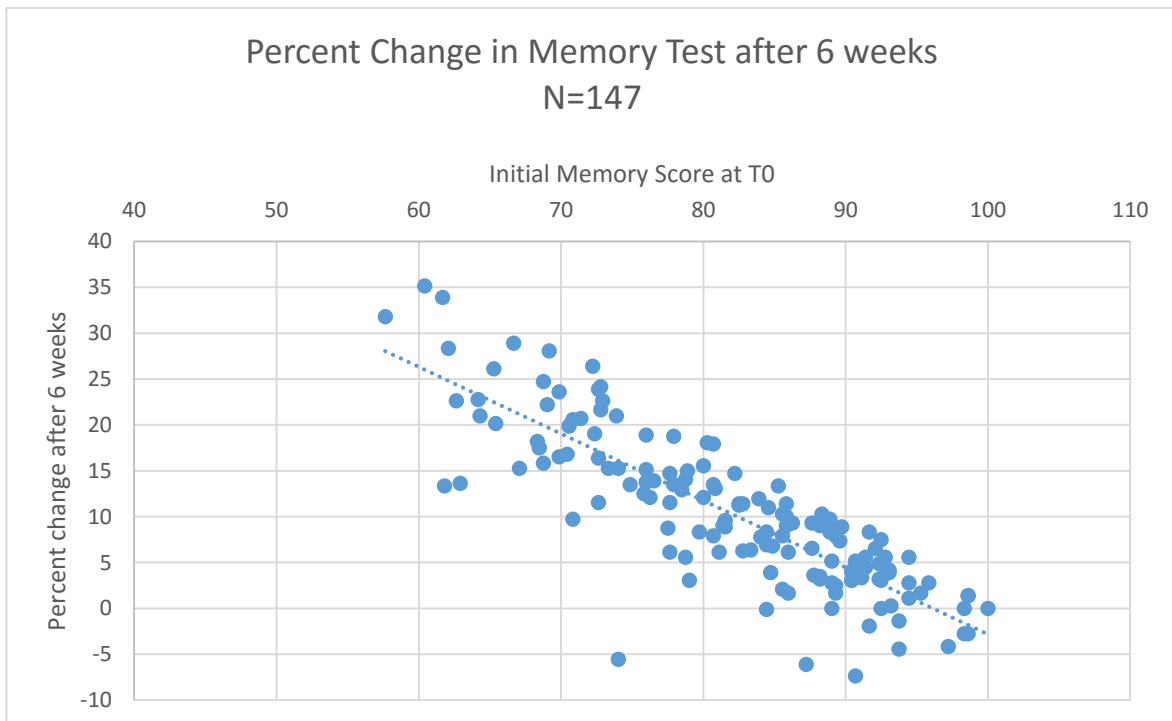
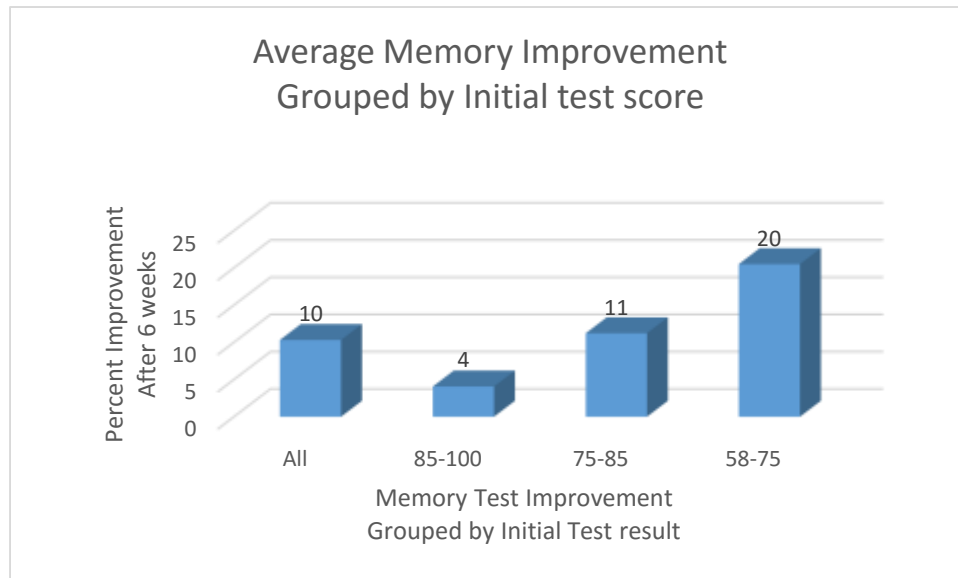


Figure 4

Non-Sequential



To determine the statistical significance of these results a number of analysis we performed. In summary, frequency of distribution of data histograms were prepared. These suggested that the data are not all normally distributed and this was confirmed by testing for normality. While the T0 data complied with the requirements for a normal distribution the later time points and in particular T6 did not. The Mann-Whitney U test was therefore chosen and the primary test for statistical significance. A student t test was also performed but in the absence of normal data the Mann Whitney U test is the most reliable. Both tests gave similar results.

The null hypothesis H (0) was that there is no significant difference between the T0 and T6 memory scores.

The alternative hypothesis H (1) is that there is a statistically significant difference between T0 and T6 memory scores. The analysis was performed using Microsoft Excel data analytical tools with the exception of the following Mann-Whitney U test formulae.

$$U_1 = R_1 - \frac{n_1(n_1 + 1)}{2}$$

$$E(U) = \frac{n_1 \cdot n_2}{2}$$

$$\sigma_U^2 = \frac{n_1 \cdot n_2 \cdot (n_1 + n_2 + 1)}{12}$$

Frequency of Data Distribution Histograms – Sequential and Non-Sequential Data

Figure 5

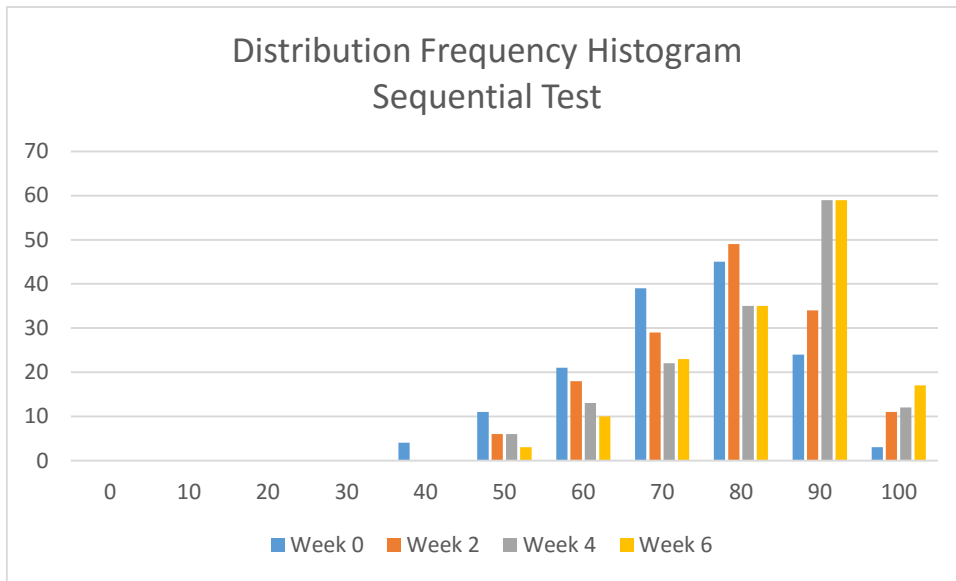


Figure 6

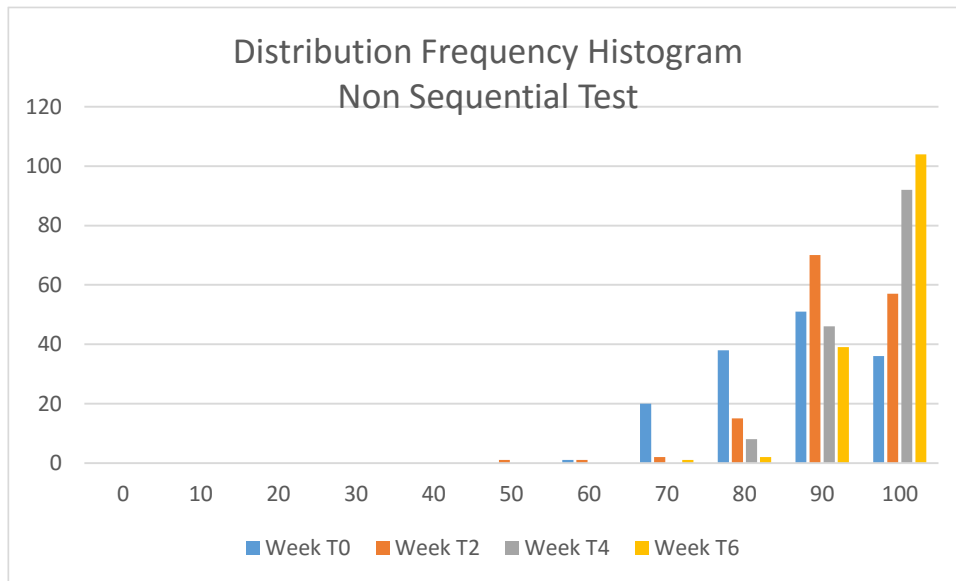


Figure 7

Mann Whitney U test Statistics Sequential Data

<i>Initial T0</i>		<i>Final T6</i>
Mean	68.66666667	80.10884
Standard Error	1.057226303	0.975429
Median	69	81
Mode	64	88
Standard Deviation	12.81818771	11.82644
Sample Variance	164.3059361	139.8648
Kurtosis	-0.06022091	-0.03107
Skewness	-0.32270495	-0.64074
Range	67	52
Minimum	33	48
Maximum	100	100
Sum	10094	11776
Count	147	147
Confidence Level(95.0%)	2.08944461	1.927784
Rank Sum	16880	26485
U	6002	15607
	6002	
mu	10804.5	
sigma	728.8492643	
p value (Mann-Whitney)	2.2117E-11	p <0.005
T.Test		
p value	8.49938E-12	p <0.005
Decision : The null hypothesis is rejected		
Conclusion: After 6 weeks there was a statistically significant increase in memory scores.		

Figure 8

Mann Whitney U test Statistics Non-Sequential Data

<i>Initial T0</i>		<i>Final T6</i>
Mean	68.66666667	80.10884
Standard Error	1.057226303	0.975429
Median	69	81
Mode	64	88
Standard Deviation	12.81818771	11.82644
Sample Variance	164.3059361	139.8648
Kurtosis	-0.06022091	-0.03107
Skewness	-0.32270495	-0.64074
Range	67	52
Minimum	33	48
Maximum	100	100
Sum	10094	11776
Count	147	147
Confidence Level(95.0%)	2.08944461	1.927784
Rank Sum	14592	28186
U	3714	17308
	3714	
mu	10804.5	
sigma	728.8492643	
p value (Mann-Whitney)	1.14134E-22	p <0.005
T.Test		
p value	1.83481E-23	p<0.005
Decision : The null hypothesis is rejected		
Conclusion: After 6 weeks there was a statistically significant increase in memory scores.		

Safety Evaluation

Anecdotally a number of participants reported other effects experienced during the trial.

Many subjects reported a general feeling of wellbeing. This effect is in line with previously reported data for Acetyl-L-Carnithine (ref 7). A number of male respondents reported and improvement in Libido similar to results seen in other trials with L-Citrulline and L-Arginine and there known effect on circulation via the Nitric Oxide pathway (ref 12). One patient reported a significant improvement in her severe arthritis but there is no way to attribute this effect to the test product.

The product was well tolerated and there were no reportable side effects recorded. Taste and palatability were considered satisfactory.

Discussion and Conclusions

This study evaluated the responses of a cohort 147 normal healthy volunteers, aged between 50 and 75, to a new functional drink containing supplements that might be beneficial to health. In particular this study measured the memory span results of the participants over a six week period. The study was not placebo controlled but the memory span test was designed to exclude 'learned' effects. After six weeks of diet supplementation with this product a highly statistically significant improvement in memory span performance was found ($p < 0.005$).

Diet supplementation with this product appears to improve memory span. Further studies are warranted to investigate potential effects on mood and / or libido.

References

1. Singh-Manoux A. et al.
Timing of the onset of cognitive decline: results from Whithall prospective cohort study.
BMJ 2012 : d7622 (1-8)
2. Holdt Levstao S. et al
Bioactive compounds in seaweed.
J Appl Phycol (2011) 23: 543 – 594
3. Rustic-Medic D. et al Eureka: Deliverable RA 2.7 – 15
Systemic review of the relationship between iodine intake and the status and developmental outcome in children.
4. Gornik H.L. and Craeger M.A.
Arginine and endothelial and Vascular Health
J Nutr 134: 2880S-2887S: 2004
5. Preli R.B. et al
Vascular Effects of dietary L-Arginine Supplementation
Arteriosclerosis, 2002 May 162(1) 1-15.
6. Seyidova et al
The role of Nitric Oxide in the pathogenesis of brain lesions.
In-Vivo 18 (3) 325 – 334.
7. Pettegrew JW et al
Acetyl-L-Carnithine physical – chemical, metabolic and therapeutic properties: relevance for its mode of action in Alzheimer's disease and geriatric depression.
Molecular Psychiatry (2000) 5, 616-632.

8. EFSA Panel on Dietetic Products, Nutrition and Allergies
Scientific Opinion on the substantiation of a health claim related to iodine and contribution to normal cognitive development pursuant to Article 14 of Regulation (EC) No 1924/2006
EFSA Journal 2014;12(1):3517
9. Wechsler, David (1939). *The Measurement of Adult Intelligence*. Baltimore (MD): Williams & Wilkins.
10. Wechsler, David (1958). *The Measurement and Appraisal of Adult Intelligence* (4th ed.). Baltimore (MD): Williams & Wilkins. Retrieved 4 June 2013.
11. Weiss, Lawrence G.; Saklofske, Donald H.; Coalson, Diane; Raiford, Susan, eds. (2010). *WAIS-IV Clinical Use and Interpretation: Scientist-Practitioner Perspectives*. Practical Resources for the Mental Health Professional. Alan S. Kaufman (Foreword). Amsterdam: Academic Press. ISBN 978-0-12-375035-8. Lay summary (16 August 2010). This practitioner's handbook includes chapters by Diane L. Coalson, Susan Engi Raiford, Donald H. Saklofske, Lawrence G. Weiss, Hsinyi Chen, Josette G. Harris, James A. Holdnack, Xiaobin Zhou, Jianjun Zhu, Jacques Gregoire, Munro Cullum, Glenn Larrabee, Gerald Goldstein, Timothy A. Salthouse, and Lisa W. Drozdick.
12. Short-term effects of L-citrulline supplementation on arterial stiffness in middle-aged men. Ochiai M, Hayashi T, Morita M, Ina K, Maeda M, Watanabe F, Morishita K. *Int J Cardiol*. 2012 Mar 8;155(2):257-61.