

Aged-garlic extract enhances global cognition

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Abstract. An epidemiological link between nitrous oxide (NO)-deficiency and Alzheimer disease has been established in preclinical models. Brain maps have revealed higher cerebral blood flow to areas of the brain involved with working or short-term memory following the consumption of NO-potent natural foods. However, aged garlic extract (AGE) may stimulate endothelial NO synthase in advance of reversing atherosclerotic plaques, which, in turn, increases NO bioavailability. The present study sought to evaluate whether AGE improves cognitive function. For this purpose, a total of 80 eligible subjects were enrolled in a prospective, randomized, placebo-controlled clinical trial, with 72 subjects completing both the baseline and the 12-week follow-up visit. The mean age of the participants was 53.0 ± 12.3 years with 45 (63%) subjects being female. The baseline characteristics, stratified by arm (AGE group, $n=37$ and the placebo group, $n=35$), of the study participants were well balanced. To assess cognition, the Montreal Cognitive Assessment (MOCA) tool was utilized. The primary endpoint was cognitive changes over the course of the study. At the conclusion of the trial, there were more patients with no cognitive impairment in the AGE arm (92 vs. 85%), similar numbers in the mild cognition arm (8 vs. 9%), and the placebo group had more patients with moderate cognitive impairment than the AGE group (6 vs. 0%). AGE had a significant effect on triglycerides; triglycerides in the AGE group decreased by 14.7 mg/dl and increased by 8.6 mg/dl in the placebo group ($P<0.05$). On the whole, in the present small study, AGE improved cognition and triglycerides. However, further larger studies with longer study durations are warranted to confirm these findings.

Introduction

The reduced bioactivity of arterial endothelial-derived nitric oxide (NO) has been demonstrated to be involved in the

pathogenesis of atherosclerosis, which leads to hypertension (1). Persistent hypertension is acutely harmful to highly vascularized organs, in particular, the brain. Endothelial dysfunction with the gradual loss of NO bioavailability in the brain is accelerated by progressive atherosclerosis, which impairs general cognitive function, but also decreases the specific cognitive domains, such as memory, visual or spatial perception and reaction time to stimuli.

An epidemiological link between hypertension induced by NO-deficiency and Alzheimer's disease has been established in preclinical models (2). Reportedly, hypertension exacerbates the Alzheimer-like pathology in amyloid protein mouse models (2), suggesting that impaired NO bioavailability contributes to the pathophysiology of Alzheimer's disease and that cognitive impairment appears to be dependent on hypertension-associated NO-deficiency.

The link between cognitive impairment and low NO bioavailability is further exacerbated by an increase in natural endothelial nitric oxide synthase (eNOS) inhibitors in pathological conditions such as hypertension, resulting in a decrease in the production of NO in the brain (3). However, the reversal of this condition by dietary NO donors has been shown. A series of studies have reported that almost 1,000 healthy seniors who had a daily helping of NO-potent foods had a slower rate of cognitive decline (4). Brain maps have revealed a higher cerebral blood flow to areas of the brain involved with working or short-term memory following the consumption NO-potent natural foods (5).

A growing number of preclinical and human studies have suggested that aged-garlic extract (AGE) possesses anti-hyperlipidemic, anti-platelet, anti-oxidative, anti-inflammatory, anti-atherosclerotic and cardio-brain protective activity, which are all likely mediated by reversing endothelial dysfunction with concomitant increase in restoring cNOS and NO bioavailability (6).

AGE appears to attenuate the progression of atherosclerotic and, independently, stimulate eNOS expression in endothelial cells, without triggering inducible NO synthase (iNOS), a potentially inflammatory response (7).

AGE may stimulate eNOS in advance of reversing atherosclerotic plaques, which, in turn, increases NO bioavailability. Therefore, AGE may restore NO bioavailability by targeting multiple levels, as follows: i) Attenuating plaque formation, thereby, reversing endothelial dysfunction, resulting in eNOS expression; ii) directly stimulating eNOS, independent of plaque

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regression; iii) inhibiting or not augmenting a NO-associated inflammatory response associated with progressive atherosclerotic lesions, i.e., AGE selectively upregulating eNOS, not iNOS.

Previous research has demonstrated an anti-hypertensive effect on both systolic and diastolic lowering with daily AGE consumption (6). The antihypertensive properties of AGE are likely the result of restoring endothelial function and stimulating eNOS, which in turn promotes vasodilation and thus, reduces blood pressure (7).

Nitric oxide generated by L-arginine metabolism by eNOS is a critical regulator of vascular function. Recent research has also shown that NO can regulate intracellular heme allocation in a concentration-dependent manner, impacting redox-sensitive proteins involved in immunity and cellular metabolism (8).

As previously mentioned, AGE may be unique among orally active 'NO boosters' since it may target both the lesion by slowing its progression and independently triggering eNOS in the endothelium despite the damage incurred by the advancing atheroma. Furthermore, it has been demonstrated *in vivo* that AGE selectively upregulates eNOS without eliciting a potentially inflammatory iNOS response (8).

A growing number of studies have demonstrated the beneficial effects of AGE on plaque progression, including increases in NO bioavailability, plaque stability, lumen diameter and fibrous cap thickness with a corresponding decrease in cholesterol crystalline, oxidized low-density lipoprotein, arterial stiffness, platelet activation among other nitric oxide-mediated outcomes (7-10). Arterial stiffness is an increasingly used metric to assess cardiovascular risk (11-15).

A growing body of evidence suggests that garlic may improve cognitive health by increasing NO bioavailability (6,16-18). Increases in NO bioavailability have been shown in multiple studies to enhance cerebral blood flow, particularly to those areas in the brain associated with working memory. A previous preclinical study suggested that garlic may improve spatial memory (18).

Materials and methods

Study design and study participants. The present study was a single-center, randomized, placebo-controlled, double-blind comparison of AGE (2,400 mg) provided by Wakunaga of America Co. Ltd. compared to matching placebo capsules for 12 weeks on endothelial function and brain function. A total of 80 eligible subjects were enrolled using at a 1:1 ratio. Subjects who met the eligibility criteria were randomly assigned to receive AGE or the placebo (cellulose based). Subjects provided written informed consent after the nature and scope of the investigation had been explained. The present study was approved by the Institutional Review Board (IRB) of the Lundquist Institute. All patients signed an informed consent prior to any study procedures being informed. The study obtained ethics approval from the WCG Institutional Review Board, study no. 132877, which acted as the central IRB.

The inclusion criteria included an age between 30-75 years, patients with pre-hypertension, 121-140 systolic blood pressure and 81-90 mmHg diastolic blood pressure or hypertension.

The exclusion criteria included any contraindication to AGE including: Any unstable psychiatric, medical, or substance abuse disorder that by the principal investigator precluded the subject's participation in the study or was likely to affect the subject's ability to complete the study, a history of malignancy within the past 5 years (other than skin cancer) or any evidence of active cancer requiring cancer chemotherapy, prior known myocardial infarction, stroke or life-threatening arrhythmia within the prior 6 months; weight in excess of 350 pounds, NYHA Class II-IV heart failure, as well as any known hypersensitivity to drugs.

Baseline information regarding risk factors for atherosclerotic cardiovascular disease (systemic hypertension, cigarette smoking, a sedentary lifestyle, a family history of premature atherosclerosis, current medications, menopausal and hormone replacement status in women, measures of obesity and chest pain questionnaire) were collected. Baseline systemic and coronary vascular function were obtained. Following randomization, participants returned at 6-week intervals to assess compliance with medication, testing and to receive an additional supply of medicine. At 12 weeks follow-up, endothelial function, inflammatory biomarkers, and repeat cognitive testing were performed.

Upon randomization into the two groups, the participants in the control and AGE group each received four capsules daily. Both the investigators and participants were blinded and medications were dispensed by a research pharmacy. Each group was randomly assigned to one of the two treatment categories in a double-blinded manner. Randomization occurred according to a computer-generated randomization code.

Study endpoints. Upon assessing cognition, the Montreal Cognitive Assessment (MOCA) tool was utilized. This is a well-validated tool, used repeatedly to assess cognitive function and changes over time. MOCA was used at week 0, and again at week 6 and week 12 (following randomization into either the placebo or AGE groups) (Fig. 1).

The domains of this test include: Visuospatial; naming; digit list; letter list; serial 7 subtraction; repeat; fluency; abstraction; delayed recall; orientation. The results were categorized as follows: 18-25 points, mild cognitive impairment; 10-17 points, moderate cognitive impairment; and <10 points, severe cognitive impairment.

Different tests will provide different insights. Of course, these types of tests can be influenced by a number of factors, including the time of day, amount of sleep, sedentary behavior vs. physically active, medical condition, emotional state and stress among other lifestyle choices, in particular dietary choices, which is no different than a blood pressure reading. Thus, all tests (baseline, 6 and 12 weeks) were administered under the same conditions, including the same room, by the same investigator and same time of day.

Statistical analysis. Data were analyzed using intention-to-treat principles, with the study subjects analyzed by treatment group assigned regardless of study drug adherence. A sensitivity analysis was performed for those adhering to interventions for >80% of the duration of the study. Differences in baseline characteristics between groups were analyzed using independent t-tests for normally distributed

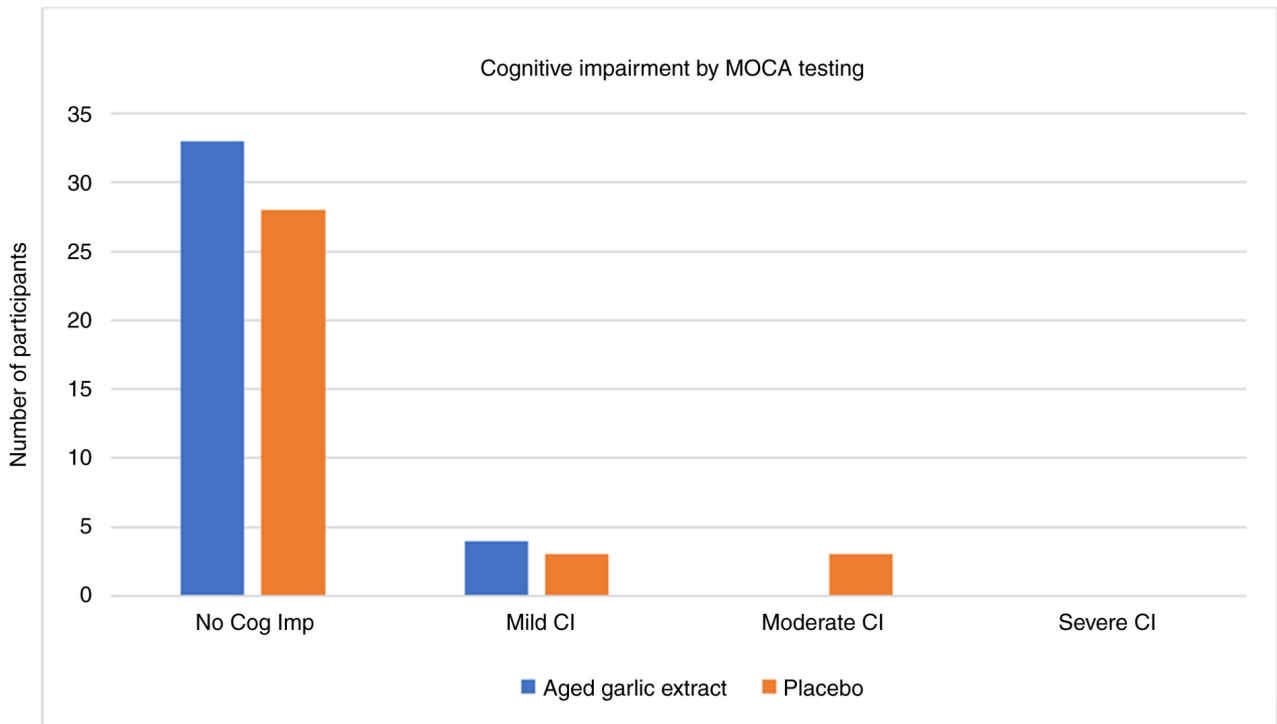


Figure 1. Results of cognitive impairment testing at follow-up in the aged garlic extract and placebo groups. MOCA, Montreal Cognitive Assessment tool; CI, cognitive impairment.

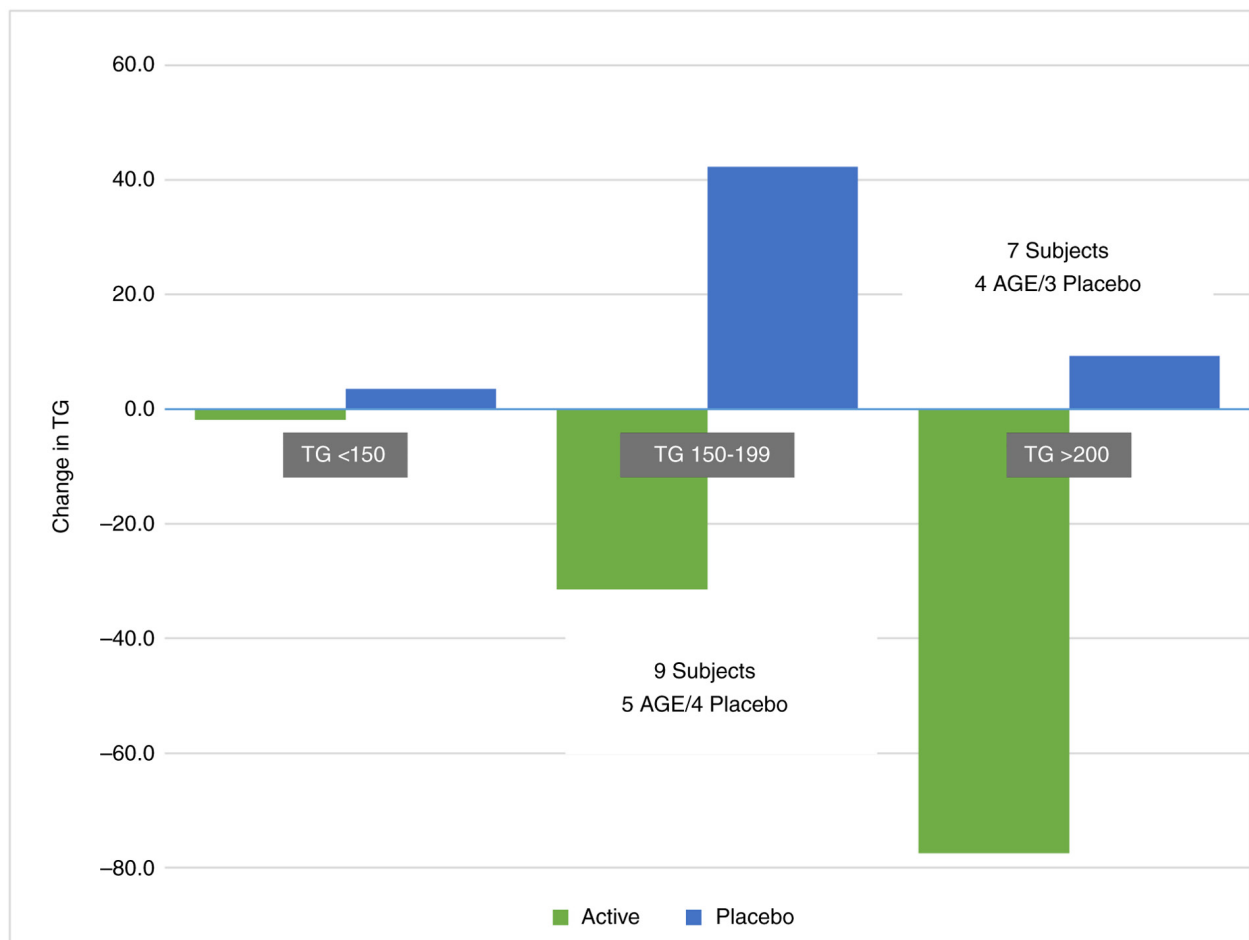


Figure 2. Change in triglycerides in the active and placebo groups. The active group refers to the aged garlic extract group. AGE, aged garlic extract; TG, triglycerides.

Table I. Baseline characteristics of the subjects in the aged garlic extract trial (n=72).

	All subjects (n=72) Mean or count (Std or %)	Aged garlic extract (n=37) Mean or count (Std or %)	Placebo (n=35) Mean or count (Std or %)	P-value ^{a,b}
Age, years	53.0 (±12.3)	51.2 (±12.4)	55.0 (±12.1)	0.194
Body mass index	31.6 (±6.9)	31.9 (±6.3)	31.2 (±7.6)	0.642
Sex				0.301
Female	45 (63%)	21 (57%)	24 (69%)	
Male	27 (38%)	16 (43%)	11 (31%)	
Heart rate	71.7 (±8.6)	72.0 (±8.1)	71.4 (±9.3)	0.771
Systolic blood pressure	130.8 (±10.5)	129.8 (±10.1)	131.9 (±11.0)	0.407
Diastolic blood pressure	82.0 (±7.0)	82.9 (±7.2)	81.0 (±6.7)	0.238
Diabetes mellitus				0.345
Yes	19 (26%)	8 (22%)	11 (31%)	
No	43 (74%)	29 (78%)	24 (69%)	
Hypertension				0.231
Yes	20 (28%)	8 (22%)	12 (34%)	
No	52 (72%)	29 (78%)	23 (66%)	
Hyperlipidemia				0.360
Yes	29 (40%)	13 (35%)	16 (46%)	
No	43 (60%)	24 (65%)	19 (54%)	
Past smoking				0.660
Yes	11 (15%)	5 (15%)	6 (13%)	
No	61 (85%)	32 (85%)	29 (87%)	
Total cholesterol	182.8 (±50.4)	189.7 (±51.0)	175.6 (±49.5)	0.246
HDL-C	53.3 (±15.7)	54.4 (±17.5)	52.1 (±13.7)	0.549
LDL-C	107.3 (±43.6)	112.3 (±46.0)	101.9 (±40.9)	0.320
Triglycerides	122.9 (±56.1)	128.6 (±52.6)	116.8 (±59.7)	0.381

^{a,b}Data presented as the mean ± SD were analyzed using an independent t-test, and data presented as numbers and percentages were analyzed using the Chi-squared test. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

continuous traits and the Chi-squared test for categorical traits. Outcomes were analyzed between the AGE and placebo group using one-way ANOVA. Least-squares means with 95% CIs and between-group differences were reported. A descriptive sub-analysis is presented with triglyceride levels categorized by <150, 150-199 and >200 mg/dl. All analyses were performed using SAS for Windows, version 9.4 (SAS Institute). All statistical tests report two-sided P-values. A P-value <0.05 was considered significant to indicate a statistically significant difference.

Results

A total of 80 subjects were enrolled in a prospective, randomized, placebo-controlled clinical trial, with 72 completing both baseline and the 12-week follow-up visit. The study was conducted according to the CONSORT (Consolidate Standards of Reporting Trials) guidelines and statement. The mean age of the participants was 53.0±12.3 years with 45 (63%) subjects being female. The baseline characteristics, stratified by arm (AGE group, n=37; and the placebo group, n=35) of the study participants were well balanced (Table I).

Table II. Final visit: Achieving maximum score per variable of the Montreal Cognitive Assessment (MOCA) tool.

Variable	Active (n=37), count (%)	Placebo (n=35), count (%)
Visuospatial (1-5)	28 (76)	18 (51)
Naming (1-3)	33 (89)	32 (91)
DigitList (0-2)	31 (84)	27 (77)
LetterList (0-1)	37 (100)	34 (97)
Serial7Subtraction (0-3)	31 (84)	30 (86)
Repeat (0-2)	35 (95)	32 (91)
Fluency (0-1)	33 (89)	27 (77)
Abstraction (0-2)	37 (100)	32 (91)
DelayedRecall (0-5)	26 (70)	20 (57)
Orientation (1-6)	36 (97)	33 (94)
MOCA total score (1-30)	IQR 30 (28,30)	IQR 28 (26,30)

The active group refers to the aged garlic extract group. MOCA, Montreal Cognitive Assessment tool.

There was a significant effect of AGE on triglyceride levels. The triglyceride levels in the placebo group at baseline were 116.8 ± 59.7 mg/dl vs. 124.9 ± 74.0 mg/dl at the study endpoint, whereas in the AGE group, baseline values were 128.6 ± 52.6 mg/dl, which decreased to 117.6 ± 46.7 mg/dl. The AGE group exhibited a mean decrease of 14.7 ± 46.4 vs. an increase in the placebo group of 8.6 ± 41.8 , for a between-group difference of 23.4 ± 44.2 mg/dl ($P=0.032$) (Fig.2).

In those with baseline triglyceride levels of ≤ 150 mg/dl [AGE group, $n=27$ (92.2 ± 26.8 mg/dl) and the placebo group, $n=25$ (119.7 ± 64.4 mg/dl)], the AGE group exhibited a decrease of -1.9 ± 37.8 mg/dl vs. an increase in the placebo group of 3.6 ± 23.3 mg/dl. In those with baseline triglyceride levels of 150-200 mg/dl [AGE group, $n=5$ (166.2 ± 12.8 mg/dl) and the placebo group, $n=4$ (172.3 ± 15.5 mg/dl)], the AGE group exhibited a decrease of -31.4 ± 26.3 mg/dl vs. increase in the placebo group.

For assessing cognition, the MOCA tool was utilized. At the conclusion of the trial, there were more patients with no cognitive impairment in the AGE arm (92 vs. 82%), similar numbers in the mild cognition arm (8 vs. 9%) and the AGE group had no patients with moderate cognitive impairment (0 vs. 9% for tge placebo). Of note, more patients had no cognitive impairment and 0 patients had moderate cognitive impairment in the AGE group. From visit 1 to the 12-week visit, MOCA increased by 3 points in the AGE group from 27 to 30, while it did not increase in the placebo group (28 to 28) (Fig. 1). A breakdown by scores of the test is presented in Table II.

Discussion

Garlic (*Allium sativum* L.) is deemed to have a variety of therapeutic applications, including platelet aggregation inhibition, the reduction of cholesterol and the control of blood pressure. These therapeutic actions of garlic parallel NO physiological effects (18). The present study demonstrated that two potential actions of NO, brachial artery reactivity and brain function, which are improved with use of AGE, substantiating earlier research on garlic with brain function and garlic with endothelial function. The present study demonstrated that AGE led to an improvement in cognitive function, as demonstrated by improvements in MOCA, as well as independent improvements in triglyceride levels. In the present small study, AGE improved cognition and triglycerides, consistent with previous research (1). AGE imparts benefits through multiple mechanisms. It has previously been demonstrated that AGE leads to improvements in lipids, inflammation and triglycerides, all of which have been shown to impact cardiovascular outcomes, including flow mediated dilation and lipid values, similar to the present study (1,19-23). AGE improved cognitive function in the present short-term (12-week) study. However, longer studies, including studies performed on individuals with more baseline cognitive impairment, are warranted.

The limitations of the present study are the small sample size, short duration (12 weeks), the use of a single cognitive tool and the lack of biomarker assessment for NO directly. Given the known benefits of AGE on hypertension, the present study opted to only include those patients with pre-hypertension. However, generalizability is limited due to the inclusion of mainly pre-hypertensive subjects. Further larger and longer studies are warranted to fully assess the physiological changes that explain cognitive improvements with AGE.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

MJB was the principal investigator. MB, HH, NK and MM assisted with the recruitment of the participants. KA, DR, TE, AG and MD assisted with the testing of the participants. AK performed the statistical analysis. All authors worked on final version and have read and approved the final manuscript. MB and AK confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board (IRB) of the Lundquist Institute. All patients signed an informed consent prior to any study procedures being informed. The study obtained ethics approval from the WCG Institutional Review Board, study no. 1328797, which acted as the central IRB.

Patient consent for publication

Not applicable.

Competing interests

Financial support was received from Wakunaga Pharmaceutical Co., Ltd.

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