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Short-term impact of aged garlic extract on endothelial function in diabetes: A randomized, double-blind, placebo-controlled trial

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Abstract. Impaired endothelial function portends an increased risk of cardiovascular disease. Vascular oxidative stress and systemic inflammation play a critical role in the pathogenesis and progression of vascular disease. Aged garlic extract (AGE) may improve impaired vascular endothelial function, while decreasing the progression of atherosclerotic plaque. We hypothesized that AGE may improve endothelial function, and in this study, we examined this hypothesis to determine whether this can be achieved over a period of 3 months, measured by the cardio-ankle vascular index (CAVI), by reducing intracellular oxidant stress and stimulating nitric oxide generation in endothelial cells. We conducted a double-blinded placebo controlled, randomized clinical trial to investigate the effects of AGE on CAVI in subjects with type 2 diabetes mellitus. A total of 65 individuals (38 men and 27 women) with a mean age of 58.8±11.1 years were enrolled and randomized to the AGE or placebo group in a double-blind placebo controlled trial. An ANOVA model with treatment as the main effect was used to compare changes in CAVI from baseline to follow-up between groups. The primary objective of this study was reduction in CAVI over a 3-month period. In the AGE group, CAVI was reduced on average by 0.71±1.27 vs. a mean reduction of 0.13±0.94 in the placebo group (P=0.04). On the whole, this study demonstrates that AGE has a positive impact on endothelial function in patients with T2DM and may play a role in the primary prevention of cardiovascular disease.

Introduction

High arterial stiffness is known to be a risk factor, as well as a prognostic marker for cardiovascular disease (CVD) (1). Endothelial dysfunction plays a key role in arterial stiffness by reducing the endothelial properties of vasodilatation, accelerating the proinflammatory and prothrombotic properties (2). Endothelial dysfunction is an early event in patients with CVD and is considered as one of the several potential contributors to plaque destabilization (3).

A number of non-invasive techniques are available to assess the endothelial function and arterial stiffness of the peripheral vasculature, such as brachial artery flow-mediated vasodilatation (bFMD), pulse wave velocity (PWV) and cardio-ankle vascular index (CAVI) (4,5). These non-invasive methods were widely used clinically to predict the risk of subclinical atherosclerosis in individuals who are at a high risk of CVD (6). CAVI is an inexpensive, non-invasive, office-based method which is used to evaluate arterial stiffness in the aorta, femoral artery and tibial artery, which reflects the degree of cardiovascular disease (1,7). The advantage to CAVI is that it is not affected by blood pressure (BP) and the measurements are automatic via validated software (6,8,9).

Our group has demonstrated the cardioprotective effects of AGE by decreasing atherosclerotic plaque progression in patients with type 2 diabetes (10) and improving endothelial function (11,12). Endothelial dysfunction plays a critical role in the pathogenesis of micro- and macrovascular diseases in patients with type 2 diabetes (13). Annual screening is recommended for diabetic patients for the early detection of micro- and macrovascular complications. This study investigated the effects of AGE in individuals with type 2 diabetes on vascular elasticity and endothelial function, which was measured by CAVI, over a period of 3 months.

Patients and methods

Study population and randomization. A total of 88 patients with type 2 diabetes were enrolled in a double-blind, placebo-controlled randomized study, who met the eligibility criteria (inclusion and exclusion criteria), after signing a written informed consent that was approved by the Institutional Review Board (IRB) of the Lundquist Institute for Biomedical Innovation at Harbor UCLA Medical Center (NCT03931434). Of these 88 patients, 23 patients were unable to undergo all follow-up visits (Fig. 1). Cardiovascular risk factors, and hemoglobin A1c (HbA1c) and serum lipid profiles were obtained using standard techniques at baseline. Participants were followed-up for 3 months with CAVI measured at baseline and again at 3 months.

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Patients were randomized at a 1:1 ratio to receive AGE (active group) or the placebo. All participants were advised to take 2 capsules twice daily with water for 3 months. The 2,400 mg of AGE capsules were provided by Wakunaga of America Co., Ltd. with a matched placebo pill which looked similar to AGE, but did not contain any garlic or active ingredient. Both the active and placebo study drugs were similar in size and color. The study drug was packaged in the containers sent by the sponsor as per the randomization list and was sent to the site with a printed label containing only the number of the patient. The randomization key was provided to the principal investigator and was opened only after the completion of the study. We conducted an inter-trial phone visit to ensure study medication compliance. The AGE capsule used in this study is commercially available in the market.

Inclusion criteria. We enrolled patients between age 30-75 years with a known history of type 2 diabetes mellitus (HbA1c >6.5% or fasting blood sugar >125 mg/dl or taking antidiabetic medications) and who signed an informed consent form.

Exclusion criteria. We excluded patients with known hypersensitivity to AGE, a body weight in excess of 350 pounds, a history of coronary artery disease (CAD), myocardial infarction (MI), stroke or life-threatening arrhythmia within the prior 6 months, New York Heart Association Functional Classification II-IV heart failure, renal impairment (serum creatinine >1.4 mg/dl), current tobacco use, a history of bleeding disorders or use of anticoagulants, hypertensive encephalopathy or cerebrovascular accident, or who were currently enrolled in another placebo-controlled trial.

Measurement of CAVI. CAVI was measured using the VaSera (Fukuda Denshi non-invasive BP, pulse wave velocity (PWV) and heart sound monitor/measuring device that integrated the values to compute an ankle-brachial index (ABI) and a proxy of arterial stiffness (CAVI). Briefly, blood pressure cuffs were applied to the bilateral upper arms and ankles, with the subject lying in the supine position and the head held in midline position. Electrocardiographic electrodes were placed on both wrists and a microphone was placed on the sternal angle for phonocardiography. After resting for 10 min, the examinations were performed. To detect the brachial and ankle pulse waves with cuffs, a low cuff pressure from 30 to 50 mmHg was used to ensure minimal effect of cuff pressure on hemodynamics. Following automatic measurements, the obtained data were analyzed using VSS-10 software (Fukuda Denshi), and the values of the right and left CAVI were measured. The averages of the right and left CAVIs were used for analysis.

Statistical analysis. Continuous variables are expressed as the means ± SD, while categorical variables are stated as counts and percentages. A Student's t-test or Chi-square test was used to determine differences in all baseline parameters between the placebo and AGE group. An ANOVA model with treatment as the main effect was used to compare changes with CAVI from baseline to follow-up between the groups, while using Tukey's procedure for post hoc analysis and comparison of multiple groups. Pearson's correlation coefficient was used to analyze the strength of the correlation between the left and right CAVI measures at each visit. A P-value of <0.05 was considered to indicate a statistically significant difference. SAS software (version 9.4) was used to carry out all statistical analyses.

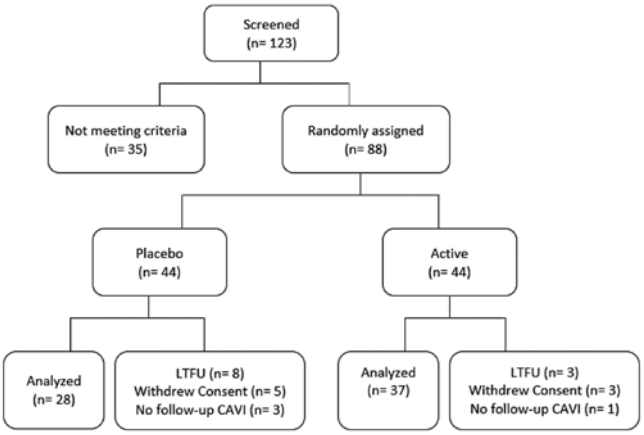

Figure 1. Flow chart of the patient cohort of this study.

Results

A total of 65 patients (38 men and 27 women; mean age 58.45±11.25 years) completed 2 study visits (baseline and 3 months), where baseline and follow-up CAVI measures were assessed (Fig. 1). Out of the 65 patients, 37 were randomized to the treatment (AGE; active) group (19 males; mean age, 59.3±10.8 years). Patients current medications, such as aspirin, hypertensive or hyperlipidemia or diabetic medications, did not change during the 3 months of the study. The baseline characteristics of the patients have been published elsewhere (14).

The primary objective of this study was reduction in CAVI over a 3-month period. As shown in Table I, CAVI was significantly reduced on the right arm by 0.64±1.09, relative to the placebo group reduction of 0.11±0.92 (P=0.04). On the left arm, CAVI was trending toward a significant reduction by 0.79±1.58, relative to 0.16±1.05 in the placebo group (P=0.07). The reduction based upon the average of both arms was 0.71±1.27 in AGE vs. 0.13± 0.94 (P=0.04). As shown Table II, the CAVI measures completed on the right and left arms for both visits were highly and significantly correlated, (Pearsons's r for visit 1 = 0.89, P<0.0001; visit 2 = 0.95, P<0.0001) indicating a high degree of consistency in the CAVI measures (Figs. 2 and 3).

Discussion

To the best of our knowledge, this is the first study to demonstrate the effects of AGE on arterial stiffness by improving endothelial function, which was measured by CAVI over a period of 3 months in patients with type 2 diabetes. Diabetic patients with a high 10-year atherosclerotic cardiovascular disease (ASCVD) risk score have been noted to have high CAVI (>9) (15). CAVI is independent of blood pressure and highly reproducible, which is significantly higher in patients with coronary artery stenosis (1,6-9,16). In comparison to PWV, CAVI reflects the smooth muscle contraction rather

Table I. CAVI index within and between both groups.

Group	Variable	Visit 1 (mean ± SD)	Visit 2 (mean ± SD)	Within group (mean ± SD)	Between group (mean ± SD)	P-value
Active (n=37)	L_CAVI	9.4±1.5	8.6±1.5	0.79±1.58	0.63±1.38	0.07
Placebo (n=28)	L_CAVI	9.2±1.5	9.0±1.4	0.16±1.05		
Active (n=37)	R_CAVI	9.3±1.4	8.7±1.4	0.64±1.09	0.53±1.02	0.04
Placebo (n=28)	R_CAVI	9.2±1.4	9.1±1.4	0.11±0.92		
Active (n=37)	Mean both CAVI	9.4±1.4	8.7±1.4	0.71±1.27	0.58±1.14	0.04
Placebo (n=28)	Mean both CAVI	9.2±1.4	9.1±1.4	0.13±0.94		

Data are presented as the means ± SD and P-values are a result of ANOVA with Tukey's test for multiple comparisons. CAVI, cardio-ankle vascular index.

Table II. Pearson's correlations of left and right CAVI measures at baseline and follow-up.

Group	Pearson's r	P-value
Visit 1, left/right	0.89	<0.0001
Visit 2, left/right	0.95	<0.0001

CAVI, cardio-ankle vascular index.

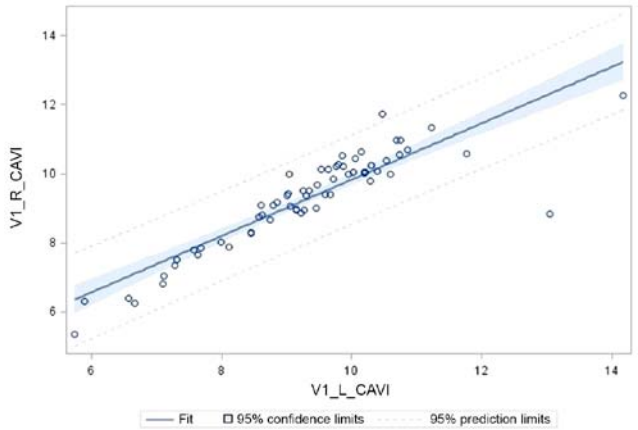

Figure 2. Fit plot of right and left CAVI at visit 1. CAVI, cardio-ankle vascular index.

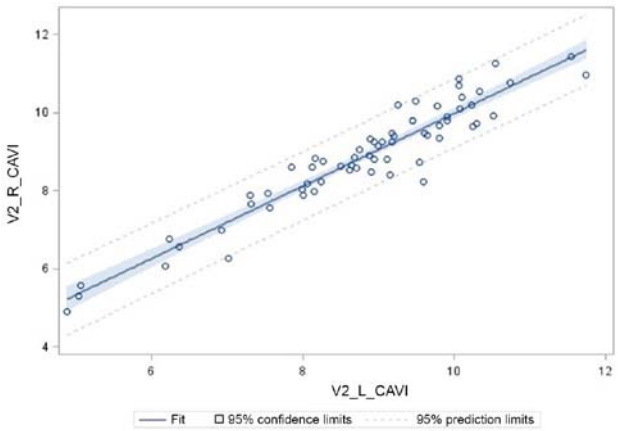

Figure 3. Fit plot of right and left CAVI at visit 2. CAVI, cardio-ankle vascular index.

than changes in BP, and changes over a short period of time in response to sympathetic tone and pharmacological influences (17). The current study demonstrated AGE reduced the arterial stiffness, which was demonstrated by CAVI, which inturn reduces the risk of coronary events.

Arterial stiffness increases with age through the loss of elastin and collagen fibers, which results in increased blood pressure (18). Kobayashi *et al* (19) demonstrated the significant association between endothelial dysfunction and increased arterial stiffness. Tomiyama *et al* (20) demonstrated the significant association of bFMD endothelial measurements with arterial stiffness assessed by PWV. Previous studies have indicated that treatments targeting to reduce arterial stiffness and wave reflections can reduce the risk of CVD along with a reduction in BP (21). Larijani *et al* (11) demonstrated the beneficial effects of AGE and CoQ10 on vascular elasticity and endothelial function in firefighters (mean reduction of PWV, 1.21; 95% CI, -2.1 to -0.32; P=0.005). Ried and Fakler (22) demonstrated the potential effect of garlic in lowering BP through a meta-analysis, including 20 clinical trials with hypertensive individuals (8-9 mmHg of SBP and 6-7 mmHg of DBP; P<0.0001). Furthermore, Breithaupt-Grögler *et al* (23) demonstrated the protective effects of garlic extract on arterial stiffness assessed by PWV (active vs. placebo; 8.3±1.46 vs. 9.8±2.45 m/sec; P<0.0001) and pressure-standardized elastic vascular resistance (EVR) (active vs. placebo; 0.63±0.21 vs. 0.9±0.44 $m^2 \cdot sec^{-2} \cdot mm\ Hg^{-1}$; P<0.0001) compared to the placebo. The results of the current study are consistent with those of previous studies and the mean of both right and left CAVI was significantly improved in AGE group relative to the placebo (0.71+1.27 vs. 0.13+0.94; P=0.04).

Oxidative stress and systemic inflammation play a key role in endothelial dysfunction, which impairs the pathways leading to the production of endothelial-derived relaxing factors, such as nitric oxide (NO), prostacyclin, tissue plasminogen activator and vasoconstrictors (eg, leukotrienes and endothelin-1) (24). Increased oxidative stress accelerates the production of reactive oxygen species (ROS), leading to the

inactivation of two anti-atherosclerotic enzymes, such as endothelial nitric oxide synthase (eNOS) and prostacyclin synthases (25,26). Endogenous NO is a potent vasodilator and is produced by two pathways. The first one is by the oxidation of L-arginine in the vascular endothelium by eNOS and the other is by reducing the dietary nitrate ($NO3^-$) to nitrite ($NO2^-$) to NO (27,28). The uncoupling of NOS is a mechanism which plays a critical role in endothelial dysfunction, resulting in the generation of high levels of superoxide ($O2^-$), leading to the formation of potent oxidant peroxynitrite ($ONOO^-$), which is highly toxic, damaging biomolecules, including proteins, lipids and DNA (22,29). Previous studies have used bFMD as an index of endothelial NO regulation of vascular tone, and targeting ROS with vitamin C and dietary nitrate supplement improved vascular function in a number of conditions known to be associated with excess oxidative stress (e.g., type II diabetes, hypertension and CAD) (30-32).

AGE contains water soluble S-allyl cysteine (SAC) and S-allymercaptocysteine (SAMC), which have potent antioxidant properties to protect the vascular endothelium from oxidative stress (11) and has also been reported to have a cholesterol-lowering effect (33). The thiol components (γ-glutamylcysteine) of garlic have the ability to reduce blood pressure by modulating NO, H2S and endothelial synthesis as previously described (21,34-36). Furthermore, AGE has been shown to exert an anti-inflammatory effect by decreasing the expression of CD36 on foam cells and oxidized LDL uptake by macrophages (37). Taken together, the antioxidant and anti-inflammatory properties of garlic increase the enzymatic activity of endothelial cells, such as eNOS, catalase, glutathione peroxidase and superoxide reductase to maintain vascular hemostasis, which may be useful for the prevention of CVD (38).

The current study has several limitations. First, the sample size was relatively small and follow-up was relatively short-term to demonstrate the effects of AGE on BP. Second, patients were under different therapies for hyperlipidemia, hypertension and type 2 diabetes mellitus at different doses. Due to our small sample size, a separate analysis by different background medications was not performed.

In conclusion, this study indicates that at the end of 3 months, the change in CAVI was significantly greater in the AGE group than in the placebo group. Further studies however, are required to evaluate whether AGE has the ability to improve arterial stiffness and endothelial function and thereby decrease adverse cardiovascular events.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article or are available from the corresponding author on reasonable request.

Authors' contributions

MJB conceived of and designed the study. SH, LC, DB, BTC and MJB collected the patient information and generated the clinical data. AK, LC, DB, SM, KS, FF, SKR and MJB analyzed and/or interpreted the data; and SH, LC, DB, AK, KS, BTC, FF and MJB drafted or revised the manuscript. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

All patients were enrolled in this study after signing a written informed consent that was approved by the Institutional Review Board (IRB) of the Lundquist Institute for Biomedical Innovation at Harbor UCLA Medical Center (NCT03931434).

Patient consent for publication

Not applicable.

Competing interests

MJB discloses work for the National Institutes of Health and General Electric Healthcare. All the other authors declare that they have no competing interests.

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