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Randomized trial evaluating the effect of aged garlic extract with supplements versus placebo on adipose tissue surrogates for coronary atherosclerosis progression

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Aims **Increased epicardial adipose tissue (EAT), pericardial adipose tissue (PAT), periaortic adipose tissue (PaAT), and subcutaneous adipose tissue (SAT) are mediators of metabolic risk, and are associated with the severity of coronary artery calcium (CAC). Aged garlic extract (AGE) has been shown to reduce the progression of coronary atherosclerosis. This study evaluates the effect of AGE with supplements (AGE + S) on EAT, PAT, SAT, and PaAT.**

Methods **Sixty asymptomatic participants participated in a randomized trial evaluating the effect of AGE + S versus placebo on coronary atherosclerosis progression, and underwent CAC at baseline and after 12 months of treatment. EAT, PAT, PaAT, and SAT volumes were measured on CAC scans. PAT was calculated as: intrathoracic adipose tissue−EAT. SAT was defined as the volume of fat depot anterior to the sternum and posterior to the vertebra. PaAT was defined as fat depot around the descending aorta.**

Results **At 1 year, the increase in EAT, PAT, PaAT, and SAT was significantly lower in the AGE + S as compared with the placebo group ($P < 0.05$). The odds ratios of increase in EAT, PAT, PaAT, and SAT were 0.63 [95% confidence interval (CI): 0.43–0.90], 0.72 (95% CI: 0.45–0.93), 0.81 (95% CI: 0.65–0.98), and 0.87 (CI: 0.52–0.98), respectively, compared with the placebo group, which even remained significant (all $P < 0.05$) after adjustment for cardiovascular risk factors and statin therapy and BMI.**

Conclusion **This study shows that AGE + S is associated with favorable effects on reducing the progression rate of adipose tissue volumes.** *Coron Artery Dis* **00:000–000**

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Keywords: adipose tissue, aged garlic extract, nonenhanced computed tomography

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Introduction

Obesity has become a widely prevalent problem in USA. Focus is being placed on obesity-related problems. Adipose tissue is recognized not merely as a passive reservoir for energy but also as a source of multiple bioactive factors. It has been shown to play an active role in atherosclerosis, plaque instability, and arterial thrombosis [1–3], and may provide as an energy reserve and protect against hypothermia [4,5]. Obesity also plays a role in insulin resistance and metabolic syndrome [6,7]. Adipose tissues may play different roles depending on location and morphology. Epicardial adipose tissue (EAT) has been shown to have both proinflammatory and anti-inflammatory roles [8], and may act as a brown adipose tissue to protect the heart against hypothermia and as an energy reserve [4,5]. Fat present around the aorta (periaortic adipose tissue, PaAT) has been shown to have an association with aortic calcifications and coronary artery calcifications (CACs) [9]. Similarly, EAT, intrathoracic adipose tissue (IAT), and subcutaneous adipose tissue (SAT) have been shown to have an association with coronary artery disease measured by CAC [9–12].

Garlic and garlic supplements (S) have been shown to have potential cardiovascular benefits by retarding the progression of coronary atherosclerosis and lowering cholesterol levels [13–17]. Aged garlic extract (AGE) has also been shown to help in lowering blood pressure, reducing platelet aggregation and adhesion, preventing low-density lipoprotein oxidation, smoking-caused oxidative damage, and also directly suppressing atherosclerosis [18–21].

Previous studies have shown an association of various adipose tissue depots with CAC in case–control studies [9–12]. We previously conducted a randomized controlled trial evaluating the effect of AGE with B-vitamins, folic acid, and L-arginine (AGE + S) on coronary atherosclerosis progression [15]. Previously, we published the effect of AGE + S on brown and white adipose tissues [22]. This study looks at the effect of AGE with B-vitamins, folic acid, and L-arginine (AGE + S) on change in intrathoracic and subcutaneous adipose tissue depots [EAT, pericardial adipose tissues (PAT), PaAT and SAT] over a 1-year period.

Methods

Study population

The participants eligible for the present study were men and women with a mean age of 60 ± 9 years (range: 40–79 years) with intermediate Framingham risk scores

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of 10–20% and CAC more than 30% [15]. The details have been mentioned elsewhere [15]. A total of 65 participants receiving chronic statin therapy were randomized to commercially available AGE + S consisting of AGE (250 mg), vitamin B6 (12.5 mg), vitamin B12 (100 μg), folate (300 μg) and L-arginine (100 mg) (Kyolic 108; Wakunaga Nutritional Supplement, Mission Viejo, California, USA) versus equivalent placebos in a double-blind manner. A total of 82 participants were screened, 65 met the eligibility criteria and were recruited, and 60 completed the study and underwent baseline and 1-year follow-up CAC scanning. Five participants did not complete the study protocol and were not included in the analysis. All participants were educated regarding a low-cholesterol diet and instructed to avoid any direct form of garlic and antioxidant supplementation. Participants with established cardiovascular disease, stroke, diabetic retinopathy, peripheral vascular disease, underlying infection, cancer, systemic inflammation status, immunosuppression, end-stage renal or liver disease, creatinine more than 1.4 mg/dl, or triglycerides more than 400 mg/dl were excluded. The BMI, hip circumference, blood pressure, fasting blood glucose, and lipid profile were obtained quarterly using standard techniques. The study protocol and consent form were approved by the institutional review board committee of our institution.

Adipose tissues measurement

Adipose tissue measurements were performed on CAC scans. The studies were performed with an E-Speed electron beam scanner (GE-Imatron, South San Francisco, California, USA). The coronary arteries were imaged with 30–40 contiguous 3-mm slices (to ensure complete coronary coverage) during end-diastole using ECG triggering during a 30–35 s breath-hold. Two experienced computed tomography readers, blinded to each other and patient characteristics, measured EAT, IAT, PaAT, and SAT. Adipose tissue was measured in axial images starting 10 mm above the superior extent of the left main coronary artery to the last slice containing part of the heart on the axial images. Volume Analysis software (GE Healthcare, Waukesha, Wisconsin, USA) was used to discern adipose tissue on the basis of a corresponding HU threshold of −190 to −30 HU (mean: −110 HU). Adipose tissue volumes were measured by a semiautomatic segmentation technique in each slice with the above display settings. The reader was required to manually trace the EAT, IAT, PaAT, and SAT. The adipose tissue volume of each fat depot is the sum of all voxels (cubic centimeters) containing adipose tissue present from 10 mm above the left main coronary artery to the last slice containing part of the heart on axial images. Adipose tissue inside the pericardial sac defined as EAT. Combined inside and outside pericardial sac adipose tissue was defined as IAT. PAT was calculated as IAT – EAT. SAT was defined as the volume of fat depot anterior to the sternum and posterior to the vertebra. PaAT was defined as volume of fat depot present around the thoracic aorta.

Statistical analysis

All statistical analyses were performed using PASW, version 18.0 (SPSS Inc., Chicago, Illinois, USA). All continuous data were presented as mean ± SD, and all categorical data were presented as percentages or absolute numbers. One-way analysis of variance was performed to assess the compatibility between two groups. Student's paired *t*-test was then performed to assess the association of changes in EAT, PAT, PaAT, and SAT over a time period of 1 year. Multiple regression analysis was performed to assess the statistical significance of association between change in AGE + S and fat depot volumes after adjustment for age, sex, diabetes mellitus, hypertension, hypercholesterolemia, family history of coronary heart disease, smoking status, statin therapy, and BMI.

Results

General characteristics of the patient population are given in Table 1. At baseline, there were no significant differences in various adipose tissue depots among AGE + S and placebo cohorts ($P > 0.05$) (Table 2). From baseline to 12 months, a significant difference was observed in the amount of adipose tissue volumes (Table 3). At 1 year, the increase in EAT, PAT, PaAT, and SAT was significantly lower in the

Table 1 Clinical characteristics of aged garlic extract plus supplements and placebo cohorts

Variables	AGE + S ($N = 33$) [*n* (%)]	Placebo ($N = 32$) [*n* (%)]	*P* value
Age [mean (SD)] (years)	60 (8)	61 (10)	0.40
Sex (male)	26 (79)	25 (78)	0.90
Hypertension[a]	18 (55)	12 (38)	0.20
Antihypertensive medications	31 (94)	32 (100)	0.85
Hypercholesterolemia[b]	24 (73)	26 (81)	0.20
Statin therapy	33 (100)	32 (100)	1.00
Diabetes mellitus[c]	1 (3)	2 (6)	0.70
Current smoker	11 (33)	12 (38)	0.70
Family history of CVD[d]	21 (64)	23 (72)	0.50
Framingham risk score 10 year risk [mean (SD)]	13 (5)	15 (7)	0.70

AGE, aged garlic extract; CVD, cardiovascular disease; S, supplements.
[a]Self-reported diagnosis of hypertension, prescribed medication for hypertension, or current blood pressure more than 140 mmHg systolic or more than 90 mmHg diastolic (>130/80 mmHg if diabetic).
[b]Self-reported diagnosis of high cholesterol, prescribed medication for high cholesterol, or current total cholesterol more than 200 mg/dl.
[c]Self-reported diagnosis of diabetes (type 1 or 2) or prescribed medication for diabetes.
[d]First-degree relative; women less than 65 years old, men less than 55 years old.

Table 2 Coronary artery calcium scores and adipose tissue volumes at baseline

Variables	AGE + S	Placebo	*P*-value
EAT	118 ± 30	110 ± 20	0.6
PAT	187 ± 53	201 ± 48	0.5
PaAT	21 ± 5	24 ± 5	0.7
SAT	45 ± 9	48 ± 8	0.8

AGE, aged garlic extract; EAT, epicardial adipose tissue; PaAT, periaortic adipose tissue; PAT, pericardial adipose tissue; SAT, subcutaneous adipose tissue; S, supplements.

AGE + S as compared with the placebo group ($P < 0.05$). The odds ratios of increase in EAT, PAT, PaAT, and SAT were 0.63 [95% confidence interval (CI): 0.43–0.90], 0.72 (95% CI: 0.45–0.93), 0.81 (95% CI: 0.65–0.98), and 0.87 (95% CI: 0.52–0.98), respectively, compared with the placebo group, which even remained significant (all $P < 0.05$) after adjustment for age, sex, diabetes mellitus, hypertension, hypercholesterolemia, family history of coronary heart disease, smoking status, statin therapy, and BMI (Table 4).

Discussion

This study shows that participants taking AGE + S had a favorable effect on EAT, PAT, PaAT, and SAT over a period of 1 year compared with placebo. The difference remained significant even after adjustment for cardiovascular risk factors and BMI. Our prior study evaluated the effect of AGE + S on CAC and white and brown EATs [22]. The brown and white EATs were defined based on different cutoffs used to define these adipose tissues (− 10 to − 87 for brown adipose tissues and − 88 to − 190 for white adipose tissues). The study showed that AGE + S was associated with a decrease in white EATs and increase in brown EAT. White adipose tissue has been shown to be associated with an increase in metabolic disease, obesity, and cardiovascular diseases, whereas brown adipose tissue is inversely associated with obesity and metabolic disease [23,24]. The current study extended this observation further evaluating the effect of AGE + S on IATs including EAT, PAT, PaAT, and SAT.

Our previous study [25] showed that EAT, PAT, and SAT are associated with the presence and severity of CAC, more so with EAT compared with PAT and SAT.

Table 3 Absolute changes at 1-year follow-up

Variables	AGE + S	Placebo	*P*-value
EAT	11 ± 8	21 ± 7	0.01
PAT	9 ± 6	19 ± 8	0.01
PaAT	2 ± 1	7 ± 1	0.01
SAT	3 ± 1	5 ± 1	0.03

AGE, aged garlic extract; EAT, epicardial adipose tissue; PaAT, periaortic adipose tissue; PAT, pericardial adipose tissue; SAT, subcutaneous adipose tissue; S, supplements.

Table 4 Odds ratios of progression of coronary artery calcification, and increase in adipose tissues in aged garlic extract plus supplements versus placebo after 1 year of treatment[a]

Model	AGE + S [OR (95% CI)]	*P*-value
Increase in EAT	0.63 (0.43–0.90)	0.02
Increase in PAT	0.72 (0.45–0.93)	0.02
Increase in PaAT	0.81 (0.65–0.98)	0.03
Increase in SAT	0.87 (0.52–0.98)	0.03

AGE, aged garlic extract; CI, confidence interval; EAT, epicardial adipose tissue; OR, odds ratio; PaAT, periaortic adipose tissue; PAT, pericardial adipose tissue; SAT, subcutaneous adipose tissue; S, supplements.
[a]Logistic regression model adjusted for age, sex, diabetes mellitus, hypertension, hypercholesterolemia, family history of coronary heart disease, smoking status, statin therapy, and BMI.

In addition, each fat depot was an independent predictor of significant CAC. Ding *et al.* [26] showed an association of IAT (EAT and PAT) with incident coronary heart disease in 998 participants of the Multiethnic Study of Atherosclerosis, which remained significant even after adjustment for BMI and cardiovascular disease risk factors. Fox *et al.* [12] showed the association of SAT with coronary artery calcium in age-adjusted and sex-adjusted models, but the association was lost after adjustment for traditional cardiovascular risk factors in 3130 participants from Framingham Heart Study who were free of clinical cardiovascular disease . Visceral adipose tissue showed an association with abdominal aortic calcification but not with SAT. This association was attenuated in multivariate analysis. This may be from the fact that SAT in association with shared cardiovascular risk factors may play a role in the development of coronary atherosclerosis. Lehman *et al.* [9] showed an association of PaAT with BMI, waist circumference, visceral adipose tissue, hypertension, low high-density lipoprotein, triglycerides, impaired fasting glucose, and diabetes. PaAT was found to be associated with abdominal aortic calcification and CAC in models including cardiovascular risk factors and visceral adipose tissue but lost significance with thoracic aortic calcification after adjustment for cardiovascular risk factors. This study shows an association of EAT, PAT, SAT, and PaAT with CAC, which remained significant even after adjustment for cardiovascular risk factors and BMI.

In addition to statin therapy, conventional therapeutic approaches have also been shown to retard the progression of CAC. Our previous study showed AGE + S to retard the progression of coronary atherosclerosis in intermediate-risk patients that remained significant even after adjustment for cardiovascular risk factors [14,15].

AGE has also been shown to help in lowering blood pressure, reducing platelet aggregation and adhesion, preventing low-density lipoprotein oxidation, smoking-caused oxidative damage, and also directly suppressing atherosclerosis [18–21] as it helps to reduce progression of atherosclerosis over a time period. However, the exact mechanism for differences in changes in adipose tissue volumes for patients with and without AGE + S supplementation is not understood.

The limitations of this study are that the study sample size was small, but we were able to show a beneficial effect of AGE + S on reduced progression of EAT, PAT, SAT, and PaAT volumes. Second, only moderate-risk individuals were included as part of the study. Third, various biomarkers secreted by adipose tissues were not studied. Fourth, it is unclear whether reduced progression of cardiac adipose tissues with AGE + S would translate into better clinical outcomes. This would require longitudinal studies looking at clinical outcomes. Finally, we were able to show the effect of AGE + S on

adipose tissue volume changes, but the exact pathophysiology of differences in patients with and without AGE + S supplementation was not studied. Further studies are warranted to look at this association in large population-based studies.

Conclusion

This study documents the beneficial effect of AGE + S on changes in EAT, PAT, PaAT, and SAT volumes over 1 year that remained significant even after adjusting for cardiovascular risk factors and statin therapy and BMI.

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Conflicts of interest

Dr Budoff is on the speaker bureau for General Electric. For the remaining authors there are no conflicts of interest.

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