International Journal of Cardiology 168 (2013) 2310–2314

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Aged garlic extract with supplement is associated with increase in brown adipose, decrease in white adipose tissue and predict lack of progression in coronary atherosclerosis

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ARTICLE INFO

Article history:
Received 29 July 2011
Received in revised form 24 November 2012
Accepted 18 January 2013
Available online 1 March 2013

Keywords:
White epicardial adipose tissue
Coronary artery calcium
Aged garlic extract
Vascular function

ABSTRACT

Background: Aged garlic extract with supplement (AGE-S) significantly reduces coronary artery calcium (CAC). We evaluated the effects of AGE-S on change in white (wEAT) and brown (bEAT) epicardial adipose tissue, homocysteine and CAC.

Methods: Sixty subjects, randomized to a daily capsule of placebo vs. AGE-S inclusive of aged garlic-extract (250 mg) plus vitamin-B12 (100 μg), folic-acid (300 μg), vitamin-B6 (12.5 mg) and L-arginine (100 mg) underwent CAC, wEAT and bEAT measurements at baseline and 12 months. The postcuff deflation temperature-rebound index of vascular function was assessed using a reactive-hyperemia procedure. Vascular dysfunction was defined according to the tertiles of temperature-rebound at 1 year of follow-up. CAC progression was defined as an annual-increase in CAC > 15%.

Results: From baseline to 12 months, there was a strong correlation between increase in wEAT and CAC ($r^2 = 0.54$, $p = 0.0001$). At 1 year, the risks of CAC progression and increased wEAT and homocysteine were significantly lower in AGE-S to placebo ($p < 0.05$). Similarly, bEAT and temperature-rebound were significantly higher in AGE-S as compared to placebo ($p < 0.05$). Strong association between increase in temperature-rebound and bEAT/wEAT ratio ($r^2 = 0.80$, $p = 0.001$) was noted, which was more robust in AGE-S. Maximum beneficial effect of AGE-S was noted with increase in bEAT/wEAT ratio, temperature-rebound, and lack of progression of homocysteine and CAC.

Conclusions: AGE-S is associated with increase in bEAT/wEAT ratio, reduction of homocysteine and lack of progression of CAC. Increases in bEAT/wEAT ratio correlated strongly with increases in vascular function measured by temperature-rebound and predicted a lack of CAC progression and plaque stabilization in response to AGE-S.

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1. Introduction

Increased regional fat distribution plays an important part in the development of an unfavorable metabolic and cardiovascular risk profile [1]. Adipose tissues (AT) are inclusive of two distinct white (WAT) and brown adipose tissues (BAT). AT is a highly metabolically active complex endocrine organ that generates various molecules with profound local and systemic effects [2,3]. The most predominant portion of AT is by far WAT, which functions to store energy in the form of triglyceride-containing intracellular droplets as well as to secrete a host of hormones and cytokines (adipokines) that regulate overall energy balance by affecting the function of other tissues including the brain, muscle, and liver [4]. The main function of BAT is to burn fat to generate heat [5,6]. Despite their similar qualitative properties, white (WAT) and brown adipose tissues (BAT) are now recognized as having distinct pro-inflammatory and anti-inflammatory functions, respectively [7–9].

Epicardial adipose tissue (EAT) is associated with multiple markers of inflammation, vascular dysfunction and oxidative stress, and is a marker for major cardiovascular events [10,7,11]. We recently reported accurate method to assess WAT and BAT using computed tomography based on Hounsfield units (HU) threshold [12].

Aged garlic extract plus supplement (AGE-S) is associated with a lack of progression of coronary atherosclerosis, improvement of vascular function and favorable effect on oxidative biomarkers [13,14]. However, the relation of AGE-S with metabolically active white (wEAT) and brown epicardial adipose tissue (bEAT) with coronary atherosclerosis is not established [15]. In this randomized study, we evaluate the effects

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0167-5273/$ – see front matter. Published by Elsevier Ireland Ltd.
http://dx.doi.org/10.1016/j.ijcard.2013.01.182

of AGE-S on change in wEAT and brown bEAT, inflammatory markers, vascular dysfunction and coronary artery calcium (CAC).

2. Methods

Sixty-five asymptomatic participants aged 40–79 years with Framingham risk scores (FRS) [16] of 10–20% and CAC > 30,who received chronic statin therapy and were free of clinical coronary artery disease (CAD), were randomized to a daily capsule of either placebo or AGE-S. AGE-S consists 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, CA, USA).

All subjects received cardio-protective lifestyle education and their digital thermal monitoring (DTM) of vascular function, homocysteine, wEAT, bEAT and CAC were measured at baseline and 12-month follows up, 60 subjects completed the study. Demographics, blood pressure and routine blood work were assessed using standard techniques. The study protocol and consent form were approved by the IRB Committee Board of the Los Angeles Biomedical Research Institute at Harbor UCLA Medical Center, Torrance, CA.

CAC scanning was performed with an E-Speed electron beam scanner (EBCT) (GE-Imatron, South San Francisco, Calif., USA). The coronary arteries were imaged with 30–40 contiguous 3 mm slices during mid-diastole using ECG-triggering during a 15 second breath hold. CAC was considered present in a coronary artery when a density of >130 Hounsfield units (HU) was detected in ≥3 contiguous pixels (>1 mm^2) overlying that coronary artery and was quantified using the previously described Agatston scoring method. CAC progression was defined as annual CAC progression > 15% [17].

Total EAT, bEAT and wEAT were measured in axial images starting 15 mm above the superior extent of the left main coronary artery (LM) to the bottom of the heart. Adipose tissue inside pericardial sac was traced manually in each slice and defined epicardial adipose tissue (EAT) [18]. Volume Analysis software (GE Healthcare, Waukesha, WI) was used to discern adipose tissue based on Hounsfield units (HU) threshold of −10 to −190 for total EAT, −10 to −87 for bEAT and −88 to −190 for wEAT [12].

Digital thermal mentoring of vascular function was measured in the morning in a quiet, dimmed room at a controlled ambient temperature of 23.5° to 25.0 °C after an overnight fast of 10 h. The measurements were obtained with the subjects in the supine position and after 30 min of rest. Subjects' blood pressure in the control arm was recorded in the sitting position 5 min before the DTM test. DTM of vascular function was obtained during 5 min of stabilization, 5 min of cuff inflation to 50 mm Hg greater than systolic blood pressure, and 5 min of deflation using an automated, operator-independent protocol (VENDYS, Endothelix, Houston, Texas). Thermal changes during the 5-minute arm cuff-induced reactive hyperemia test were monitored continuously in the fingertip using VENDYS software. The device consists of a computer-based thermometry system (0.006 °C thermal resolution) with fingertip resistance temperature detector fast response probes designed to minimize the skin-probe contact area and fingertip pressure that attach to the pulp of the index finger on hand. The system includes a common automated sphygmomanometer cuff, cuff-inflation pump, and release valve to permit noninvasive measurement of the arterial pressure and the control of occlusive hyperemia. Dual-channel temperature data are simultaneously acquired at a 1-Hz sampling rate. Vascular function was measured based on the amount of temperature rebound (TR) in the fingertip during the reactive hyperemia procedure.

2.1. Statistical analysis

All statistical analyses were performed using SAS 9.2 (www.sas.com, Cary, NC) and STATA 12.1(www.stata.com, College Station, Texas). All continuous data are presented as a mean value ± SD, and all categorical data are reported as a percentage or absolute number. Student's *t* tests and Chi-square tests were used to assess differences between groups. Logistic regression analyses were employed to assess the change in total EAT, bEAT, wEAT, homocysteine, vascular function and coronary atherosclerosis in response to AGE-S. These analyses were adjusted for demographics, age, gender, conventional cardiovascular risk factors, body mass index and statin therapy.

3. Results

Table 1 shows that there were no significant differences between groups in age, gender, and traditional cardiovascular risk factors at baseline. Table 2 demonstrates the baseline, 1-year follow-up and annual change in CAC, homocysteine, temperature rebound, total EAT, bEAT and wEAT. There were no significant differences between groups in CAC, homocysteine, temperature rebound, wEAT, bEAT and total EAT at baseline ($p > 0.05$). At 1 year, increases in CAC, total and white EAT were significantly lower in AGE-S as compared to placebo ($p < 0.05$). In addition, temperature rebound, levels of brown EAT and bEAT/wEAT ratio were significantly higher in AGE-S as compared to placebo ($p < 0.05$). Similarly, levels of homocysteine were significantly lower in AGE-S ($p < 0.05$) (Table 2).

From baseline to 12 months, there was a strong correlation between increase in wEAT and CAC ($r^2 = 0.54$, $p = 0.0001$) as demonstrated in Fig. 1. The increase in bEAT correlated directly with decreases in homocysteine levels ($r^2 = 0.66$, $p = 0.0001$). Strong association between increase in temperature rebound and bEAT/wEAT ratio ($r^2 = 0.80$, $p = 0.001$) was noted, which was more robust in AGE-S (Fig. 2).

Table 1
Baseline demographic characteristics of study subjects.

Variable	AGE-S n = 33	Placebo n = 32	p
Baseline			
Age (years)	60 (8)	61 (10)	0.40
Gender (% male)	26 (79%)	25 (78%)	0.90
Hypertension	18 (55%)	12 (38%)	0.20
Antihypertensive medication (%)	31 (94%)	32 (100)	0.85
Hypercholesterolemia (%)	24 (73%)	26 (81%)	0.20
Diabetes Mellitus (%)	1 (3%)	2 (6%)	0.70
Current smoker (%)	11 (33%)	12 (38%)	0.70
Family History of CAD	21 (64%)	23 (72%)	0.50
Framingham risk score (%)	13 ± 5	15 ± 7	0.7

Table 3 reveals that the risk of CAC progression was 65% less with AGE-S as compared to placebo ($p = 0.03$). After adjustment for risk factors, the relative risk of each standard deviation increase in total EAT and wEAT was 0.63 (95% CI 0.43–0.90, $p = 0.02$) and 0.43 (95% CI 0.25–0.75, $p = 0.003$) in AGE-S as compared to placebo. The risk of each standard deviation increase in homocysteine was 0.63 (95% CI 0.43–0.91, $p = 0.01$) in AGE-S as compared to placebo. In addition, risk of each standard deviation increase in temperature rebound, bEAT and bEAT/wEAT ratio was 94%, 101% and 189% higher in AGE-S as compared to placebo ($p < 0.05$).

Fig. 3 shows that annual percent CAC increased proportionally with decrease in bEAT/wEAT ratio as well as decrease in temperature rebound ($p < 0.05$). Maximum beneficial effect of AGE-S was noted with increase in bEAT/wEAT ratio, temperature-rebound, and lack of progression of wEAT and CAC. The likelihood ratio of combined lack of progression in CAC and increase in bEAT/wEAT ratio and temperature rebound was 12.82 (95% CI 4.05–41.66, $p = 0.0001$) in AGE-S as compared to placebo.

4. Discussion

The current study demonstrates that: 1) AGE-S is independently associated with reduction of homocysteine wEAT and progression of CAC, 2) AGE-S is independently associated with increase in bEAT, bEAT/wEAT ratio and temperature rebound, 3) a strong direct relationship between the increase in wEAT and progression of coronary atherosclerosis exists, and 4) increase in bEAT and bEAT/wEAT ratio was associated with increases in vascular function measured by temperature rebound, similar to previous studies which increase in temperature rebound has been associated with evidence of concomitant removal of OxPL from the vessel wall and plaque stabilization in animal models, and predicted lack of progression of CAC [19].

Progression of CAC, measured by cardiac CT, is a marker of cardiovascular prognosis even after adjustment for the extent of baseline CAC [17]. Previous studies have shown that the progression of CAC, defined as clinically significant when the rate of change exceeds 15% per year based upon clinical outcomes studies, may provide incremental prognostic information beyond that provided by the baseline calcium score itself [17]. We recently reported that EAT is independently associated with the presence and severity of CAC after adjustment for age, gender, BMI, and cardiovascular risk factors [18]. Ding et al. [20] showed that there is an independent association of EAT with incident coronary artery disease in 998 participants of the Multiethnic Study of Atherosclerosis. Increases in total EAT is associated with increased inflammation, insulin resistance, dyslipidemia, obesity, cardiovascular risk factors, metabolic dysfunction, and coronary atherosclerosis [21].

Table 2
Baseline, 1-year follow-up and annual change in different adipose tissues, temperature rebound, homocysteine and coronary artery calcium.

Variable	Baseline			1-year follow up			Annual absolute change		
	AGE-S	Placebo	p	AGE-S	Placebo	p	AGE-S	Placebo	p
CAC (AJ)	291 ± 50	347 ± 67	0.3	311 ± 48	439 ± 77	0.03	22 ± 18	62 ± 43	0.005
Total EAT (cc)	118 ± 30	110 ± 20	0.6	127 ± 41	135 ± 48	0.04	11 ± 8	21 ± 7	0.01
wEAT (cc)	79 ± 45	77 ± 33	0.8	83.8 ± 25.9	100.9 ± 29.6	0.001	4.7 ± 16.6	24.5 ± 12.9	0.0001
bEAT (cc)	38 ± 15	36 ± 13	0.5	43.4 ± 15.9	33.7 ± 13.89	0.001	4.5 ± 3.4	−2.8 ± 2.4	0.0001
bEAT/wEAT ratio	48 ± 18%	52 ± 21%	0.3	60 ± 19%	39 ± 20%	0.001	125 ± 38%	−11 ± 20%	0.0001
Temperature rebound	0.57 ± 0.2	0.56 ± 0.15	0.9	1.36 ± 0.2	0.71 ± 0.2	0.04	0.79 ± 0.2	0.10 ± 0.2	0.001
Homocysteine, mg/dL	10.1 ± 2.4	10.7 ± 2.2	0.3	8.3 ± 2.7	10.6 ± 2.6	0.02	−1.7 ± 2.5	−0.2 ± 2.4	0.004

The current study showed that AGE-S is associated with decrease in progression of EAT and CAC, also revealed significant direct correlation between change in wEAT and CAC.

Unlike WAT which is associated with increase in metabolic disease, obesity and cardiovascular diseases, expansion of BAT resulted in opposite effects on body weight and metabolism [22]. In which, brown adipose tissue (BAT) is inversely associated with obesity and metabolic disease [23]. In rodent models several pharmacological approaches which increase BAT activity, have been proven to effectively prevent obesity, facilitate weight reduction, and ameliorate insulin resistance [24]. Brown fat cells could derive from direct conversion of white adipocytes [25–27]. In response to cold, appearance of brown fat cells is observed in mouse visceral WAT [1]. The study of modified human subcutaneous white adipocytes to express human PPARγ coactivator 1α (PGC-1α), as opposed to intact white adipocytes with low expression of low levels of PGC-1α, demonstrated an increase in the capacity of fatty acid oxidation as well as expressed higher level of nuclear receptor, peroxisome proliferator-activated receptor α (PPARα), in modified white adipocytes similar to BAT [28–31]. Previous studies revealed that PPARα is involved in the remodeling of WAT of mice treated with a β3-adrenergic agonist with appearance of brown fat-like adipocytes [32]. The current study reconfirms previous studies and demonstrates increase in brown to white epicardial adipose tissue in response to AGE-S.

Previous studies have demonstrated that impaired vascular function is associated with higher risk of subsequent atherosclerotic cardiovascular disease events [33]. Similarly, several studies have demonstrated strong correlations between vascular dysfunction and cardiovascular risk factors and CAC [34,35]. Nevertheless, there is considerable heterogeneity in the magnitude of vascular dysfunction in individuals with similar risk factor profiles [36]. In this regard, vascular dysfunction may be seen as an important "integrative factor" of the inherent atherosclerotic risk of an individual which takes into account the cumulative effects of various risk and protective factors [36]. In addition to risk assessment for prediction of clinical outcomes measures of vascular function may be used to evaluate response to therapies [37,38].

We recently reported that increases in CAC were inversely correlated with the increases in vascular function and OxPL/apoB and Lp (a) levels in response to AGE [14]. The findings of the current study reveal direct correlation between changes in bEAT/wEAT ratio with increase of vascular function in response to AGE-S. These salutary effects have been associated with evidence of concomitant removal of oxidized phospholipids from the vessel wall and the stabilization of atherosclerosis [39], as well as positive changes in bEAT/wEAT ratio [40], and correlate strongly with increases in vascular function and regression in the burden of atherosclerosis [14].

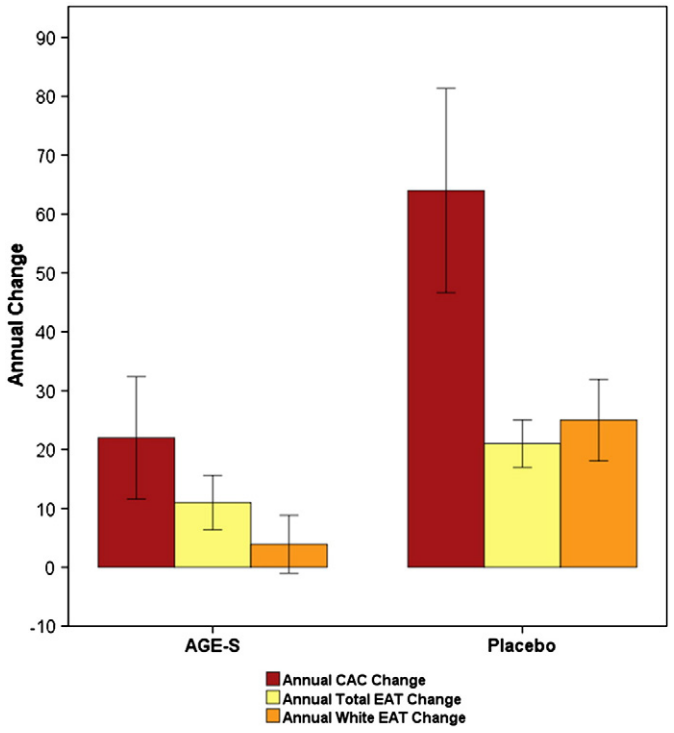

Fig. 1. Aged garlic extract plus supplement is associated with significant decrease in the progression of coronary artery calcium and epicardial adipose tissue (EAT), especially white EAT.

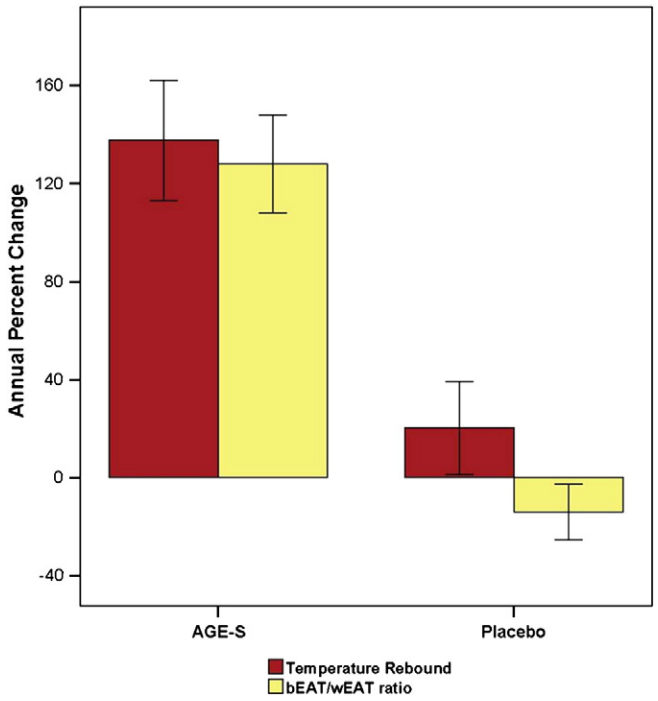

Fig. 2. Aged garlic extract plus supplement is associated with significant increase in temperature rebound and brown to white epicardial adipose tissue ratio.

Table 3
The effect of AGE-S on various epicardial adipose tissues, temperature rebound, homocysteine and coronary atherosclerosis.

Model	Placebo	Aged garlic extract OR (95% CI)	p
CAC progression[†]	1.0 (Ref)	0.35 (0.1–0.85)	0.03
Increased tEAT[Δ]	1.0 (Ref)	0.63 (0.43–0.90)	0.02
Increased wEAT[Δ]	1.0 (Ref)	0.43 (0.25–0.75)	0.003
Increased bEAT[Δ]	1.0 (Ref)	2.01 (1.24–3.45)	0.001
Increased bEAT/wEAT[Δ]	1.0 (Ref)	2.89 (1.67–4.98)	0.0001
Increased temperature rebound[Δ]	1.0 (Ref)	1.94 (1.32–2.84)	0.001
Increased homocysteine [Δ]	1.0 (Ref)	0.63 (0.43–0.91)	0.01

Logistic regression analysis.
Adjusted for age, gender, diabetes mellitus, hypertension, hypercholesterolemia, family history of CHD, smoking status, statin therapy and BMI.
[†] Relative risk of CAC progression (increase in CAC ≥ 15%/year).
[Δ] Relative risk of each standard deviation increase in total (tEAT), brown (bEAT) and white epicardial adipose tissue (wEAT) as well as temperature rebound and homocysteine.

Hyperhomocysteinemia is associated with insulin resistance and endothelial cell injury through the induction of resistin expression and secretion from adipocytes via the activation of the reactive oxygen species, protein kinase C, and nuclear factor kappaB pathway [41–43]. Furthermore, aggregation of lipoproteins complexed with homocysteinylated and oxidized lipoproteins, and lipoprotein autoantibodies in areas of high tissue pressure, causing ischemia, vascular dysfunction and plaque vulnerability [44]. This study confirms previous studies and provided evidence of linkage of increase in bEAT/wEAT ratio and increase in vascular function with decrease in inflammation measured by homocysteine in response to AGE-S which predicts lack of CAC progression and plaque stabilization.

5. Limitations

This study has several limitations. The study sample size was small; however this study clearly demonstrates the beneficial effects of AGE-S on total EAT, wEAT, bEAT and inflammatory biomarkers and CAC progression. Further studies are needed to assess the long-term effect of AGE-S on different adipose tissues on major adverse cardiovascular events (MACE).

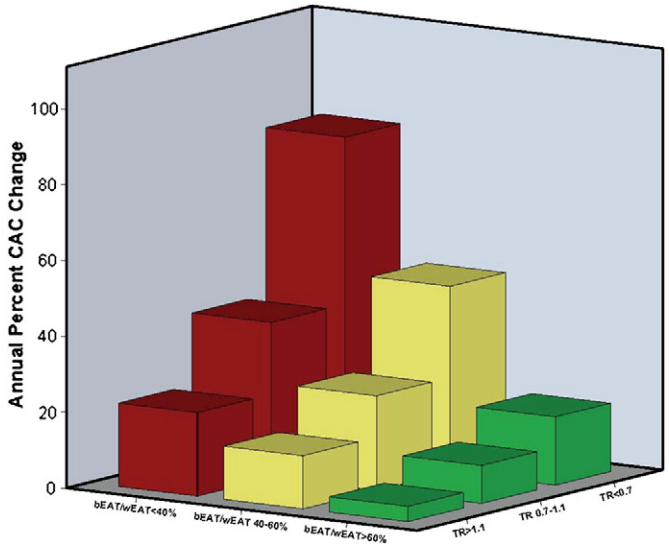

Fig. 3. Annual percent coronary artery calcium increased proportionally with decrease in brown to white epicardial adipose tissue ratio as well as increase in temperature rebound.

6. Conclusion

AGE-S is associated with reduction of homocysteine, wEAT and progression of CAC. Additionally, increases in bEAT/wEAT ratio in response to AGE-S were directly correlated with the increase in vascular function measures by temperature rebound and decrease in homocysteine, and were associated with the lack of CAC progression; highlighting the important role of conversion of wEAT to bEAT with improvement of vascular function and lack of progression of CAC.

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