

## Independent beneficial effects of aged garlic extract intake with regular exercise on cardiovascular risk in postmenopausal women

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### Abstract

The purpose of the study was to assess the effects of a 12 weeks aged garlic extract (AGE) regimen with regular exercise on cardiovascular disease (CVD) risk in postmenopausal women. A total of 30 postmenopausal women (54.4 ± 5.4 years) were randomly divided into the following four groups: Placebo (Placebo; n = 6), AGE intake (AGEI; n = 8), exercise and placebo (Ex + Placebo; n = 8), exercise and AGE (Ex + AGE; n = 8) groups. The AGE group consume 80 mg per day, and exercise groups performed moderate exercise (aerobic and resistance) three times per week. After 12 weeks of treatment, body composition, lipid profile, and CVD risk factors were analyzed. Body weight was significantly decreased in AGEI, Ex + Placebo, and Ex + AGE groups compared to baseline. Body fat % was significantly decreased in the AGEI and Ex + Placebo groups. Body mass index (BMI) was significantly decreased in the AGEI, Ex + Placebo, and Ex + AGE groups. Fat-free mass was significantly decreased in the AGEI group. Total cholesterol (TC) was significantly lower in the Ex + Placebo compared to the Placebo group. AGE supplementation or exercise effectively reduced low-density lipoprotein (LDL-C). Triglyceride (TG) was significantly increased in the AGEI group. Malondialdehyde (MDA) levels were significantly decreased in the AGEI, Ex + Placebo, and Ex + AGE compared to the placebo group. AGE supplementation reduced homocysteine levels regardless of whether the women also exercised. The present results suggest that AGE supplementation reduces cardiovascular risk factors independently of exercise in postmenopausal women.

**Key Words:** Aged garlic extract, exercise, postmenopausal women, cardiovascular risk factor, homocysteine

### Introduction

Menopause is a well-known risk factors for cardiovascular disease (CVD) and leads to increased metabolic parameters [1,2]. The evidence shows that changes in the reproductive hormones affect vascular function in postmenopausal women [3,4]. Other CVD risk factors include excessive weight, diabetes mellitus, dyslipidemia, hypertension, endothelial dysfunction and physical inactivity [5-9], which are associated with impaired cardiac function, dysregulated lipid metabolism, and increased oxidative stress level [10-12].

A primary preventative measure for postmenopausal women with increased CVD risk factors is exercise training with or without diet interventions [13,14]. Duvernoy *et al.* [15] and Yoshizawa *et al.* [16] demonstrated that regular exercise significantly improved physical fitness and attenuated CVD risk

factors in postmenopausal women. Moreover, previous studies have demonstrated that regular exercise training enhances endothelial function, which has profound stimulatory effects on key vasodilatory enzymes, such as nitrite/nitrate, endothelin-1, and homocysteine [17-19].

Several evidence suggests that nutrition is very important for decreasing CVD risk factors in postmenopausal women, and phytochemical agents found in herbs and plants can be beneficial effect [20-22]. Garlic has been used to treat hypertension, coronary diseases, and CVD, in adult since ancient times [23,24]. Aged garlic extract (AGE) is an odorless product that is made from garlic that has been aged at room temperature. It is bioavailable in animals and humans following oral administration [23-25]. Previous studies have demonstrated that AGE reduces lipid cholesterol, oxidative stress, blood pressure, and improves endothelial function [26-30]. However, the basic mechanisms

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behind these positive effects are not fully understood in postmenopausal women, and the effects of combined intervention with exercise are largely unknown. Accordingly, we hypothesized that combined intervention may reduce CVD risks to a greater extent than mono-therapy with either treatment.

Therefore, we evaluated the effects of a 12 week AGE regimen with or without regular exercise on body composition, lipid profile, and CVD risk factors in postmenopausal women.

## Subjects and Methods

### *Participant recruitment*

Women were eligible if they had experienced menopause, which was defined as the absence of menstruation for at least 1 year, and had not received hormone therapy (HT) for the past 6 months. Study participants were recruited from the general population through advertisements and the local public health center. Exclusion criteria included CVD, pulmonary, or metabolic disease (hypertension, dyslipidemia, diabetes, etc.), orthopedic problems, and smoking. Before participation, all subjects provided written informed consent on an acknowledgment form approved by the institutional human research committee.

A total 33 of apparently healthy, sedentary postmenopausal women participated in this study. Three participants were excluded due to a diagnosis of hypertension. Subjects ( $n = 30$ , age:  $54 \pm 5.4$ , body weight:  $60.2 \pm 6.7$  kg, body mass index (BMI):  $24.0 \pm 2.4$ ) were randomly divided into the following interventions: placebo group (Placebo;  $n = 6$ ), AGE intake group (AGEI;  $n = 8$ ), exercise and placebo group (Ex + Placebo;  $n = 8$ ), exercise and AGE group (Ex + AGE;  $n = 8$ ).

### *Study design*

Each subject reported to the laboratory at 9:00 a.m. following an overnight fast on two separate occasions (pretraining, posttraining) for blood sampling. Participants were asked not to consume caffeine or alcohol for at least 12 hours and not to perform exercise training for 48 hours before the measurement. Participants rested for a minimum of 10 minutes before their blood pressures was measured over brachial artery using a semiautomated device (VP-1000; Colin Medical Technology, Komaki, Japan) while they were in a supine position.

The Korean Garlic Corporation (Ulsung, Korea), provided bioavailable standardized AGE supplements. AGE was provided in packs containing 80 ml of AGE, which was derived from organically grown garlic, and also contained sulfide, disulfide, S-allylcysteine (SAC), and protein. Concentrated AGE was further diluted to 65 brix with distilled water. Placebo packs were matched to the active pack with regard to shape. AGE and placebo intake groups consumed one pack five times a week for 12 weeks.

Exercise training groups trained three times per week on

nonconsecutive days during the study period. The training program consisted of two mixed activities (aerobic and resistance) as previously described [31]. The exercise intensities for resistance and aerobic training were adjusted to keep the heart rate (HR) at 60% of the predicted maximum HR throughout the study. Exercise training was supervised by a professional educator. Exercise HRs was measured using a polar device (Electro; Oy, Kempele, Finland).

### *Blood collection*

Blood was collected in vacutainer (BD Bioscience, San Jose, CA, USA) from the antecubital vein of the arm following an overnight fast. After centrifugation at  $1500 \times g$  for 15 min, separated serum and plasma were stored in multiple aliquots at  $-80^\circ\text{C}$  until they were assayed. After completion of the protocol, samples from each subject were analyzed in the same group to minimize laboratory variability.

### *Anthropometric and biomedical measures*

Body composition, blood pressure and blood sample were evaluated before and 12 weeks. Body composition was measured with a pristine analyzer that uses multi frequency electric impedance to assess weight, fat percentage, and fat-free mass (Jawon Medical, Plus 720). BMI was calculated using the equation of weight (kg)/height ( $\text{m}^2$ ).

Systolic and diastolic BP (SBP and DBP, respectively) were measured using a semiautomated device (VP-1000; Colin Medical Technology, Komaki, Japan) with subjects in the supine position. BP was assessed twice, and then the average of the two values was used for subsequent analyses. Mean arterial pressure (MAP) was calculated as  $\text{MAP} = (1/3) \text{SBP} + (2/3) \text{DBP}$ ; pulse pressure (PP) was calculated as  $\text{PP} = \text{SBP} - \text{DBP}$ .

Fasting blood samples were taken from antecubital vein. Serum total cholesterol (TC), triglyceride (TG), and high-density (HDL-C) and low-density (LDL-C) lipoprotein levels were measured using enzymatic techniques based on colorimetric measurement with Bayer kits (ADVIA 1650, Japan). The plasma level of malondialdehyde (MDA; Oxis, USA), endothelin-1 (ET-1; R & D Systems, USA) was measured using an enzyme-linked immunosorbent assay (ELISA) method. Nitrite/nitrate (R & D Systems, USA) were measured using an enzyme immunoassay (EIA) method. Insulin (Roche, Germany) was measured using an enhanced chemiluminescence assay (ECLIA) method. Homocysteine (ABBOTT, USA) was measured using a carbonyl-metalloimmunoassay (CMIA) method. All assays were performed according to manufacturers' instructions.

### *Statistical analysis*

To determine the effect of each intervention on all data, repeated-measures analysis of variance (ANOVA) was used.

When indicated by a significant main effect or interaction, specific mean comparisons were performed to identify significant differences within each intervention. In the case of a significant F value, a post hoc test using Tukey's least significant difference (LSD) method was used to identify significant differences among mean values. All data were reported as means  $\pm$  standard deviation (SD). Statistical significance was set at  $P < 0.05$ . Statistical analyses were performed using SPSS, version 19.0 (SPSS, Chicago, IL).

## Results

### Anthropometry

The study included 30 Korean women  $54.4 \pm 5.4$  years of age who were classified as postmenopausal and had not had menstrual cycle for at least 1 year. There were no differences in baseline

parameters between the groups. Participant characteristics at baseline and after AGE intervention are presented in Table 1.

Following exercise and AGE intervention, body weight was significantly decreased in the AGE, Ex + Placebo ( $P < 0.001$ ), and Ex + AGE ( $P < 0.01$ ) groups. Body fat % was significantly decreased in AGE ( $P < 0.001$ ), and Ex + Placebo ( $P < 0.01$ ) groups. BMI was significantly decreased in AGEI ( $P < 0.001$ ), Ex + Placebo ( $P < 0.01$ ), and Ex + AGE ( $P < 0.5$ ) groups. Fat-free mass was significantly decreased in the AGEI group ( $P < 0.01$ ). However, there were no statistically significant body composition changes among the groups.

### Lipid metabolism

After 12 week, TC was significantly lower in the Ex + Placebo group compared the Placebo group ( $P < 0.01$ ). TC levels slightly decreased with intervention, but the difference was not

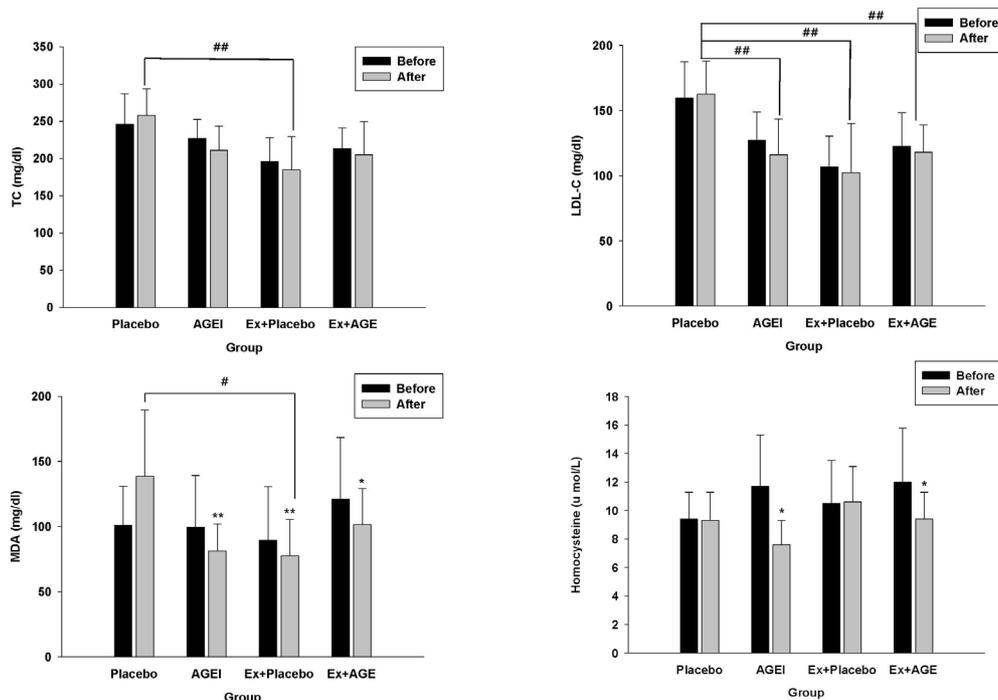
**Table 1.** Participant characteristics at baseline and after the exercise and AGE intervention in postmenopausal women<sup>1)</sup>

|                          | Placebo (n = 6) |                 | AGEI (n = 8)    |                   | Ex + Placebo (n = 8) |                   | Ex + AGE (n = 8) |                  |
|--------------------------|-----------------|-----------------|-----------------|-------------------|----------------------|-------------------|------------------|------------------|
|                          | Before          | After           | Before          | After             | Before               | After             | Before           | After            |
| Age (yrs)                | 54.4 $\pm$ 5.4  | -               | 51.8 $\pm$ 1.7  | -                 | 54.8 $\pm$ 7.7       | -                 | 56.2 $\pm$ 6.2   | -                |
| Height (cm)              | 155.2 $\pm$ 6.1 | -               | 159.2 $\pm$ 6.3 | -                 | 160.1 $\pm$ 4.8      | -                 | 158.0 $\pm$ 5.7  | -                |
| Weight (kg)              | 61.7 $\pm$ 7.5  | 59.8 $\pm$ 7.8  | 58.3 $\pm$ 5.2  | 55.3 $\pm$ 5.2*** | 62.1 $\pm$ 8.6       | 60.2 $\pm$ 8.1*** | 59.2 $\pm$ 5.9   | 57.8 $\pm$ 6.1** |
| Body fat (%)             | 33.9 $\pm$ 1.5  | 32.4 $\pm$ 0.4* | 29.4 $\pm$ 5.0  | 27.5 $\pm$ 5.1*** | 31.0 $\pm$ 3.4       | 29.8 $\pm$ 3.1**  | 30.36 $\pm$ 3.6  | 29.6 $\pm$ 3.1   |
| BMI (kg/m <sup>2</sup> ) | 25.5 $\pm$ 1.4  | 24.9 $\pm$ 1.3  | 23.1 $\pm$ 3.1  | 21.8 $\pm$ 2.9*** | 24.1 $\pm$ 2.5       | 23.2 $\pm$ 2.4*** | 23.6 $\pm$ 1.7   | 23.2 $\pm$ 1.5*  |
| FFM (kg)                 | 37.3 $\pm$ 5.1  | 37.0 $\pm$ 5.1  | 37.6 $\pm$ 2.1  | 36.7 $\pm$ 2.3**  | 39.0 $\pm$ 3.6       | 38.6 $\pm$ 3.6    | 37.7 $\pm$ 3.5   | 37.3 $\pm$ 3.5   |

<sup>1)</sup> Values are means  $\pm$  SD.

BMI, body mass index; FFM, fat-free mass

\* $P < 0.05$  vs before intervention, \*\* $P < 0.01$  vs before intervention, \*\*\* $P < 0.001$  vs before intervention.



**Fig. 1.** Effect of exercise and AGE intervention on blood markers in postmenopausal women. TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; MDA, malondialdehyde. # $P < 0.05$  vs placebo, ## $P < 0.01$  vs placebo, \* $P < 0.05$  vs before intervention, \*\* $P < 0.01$  vs before intervention.

**Table 2.** Effect of exercise and AGE intervention on markers of lipid metabolism in postmenopausal women<sup>1)</sup>

|               | Placebo (n = 6) |               | AGEI (n = 8) |             | Ex + Placebo (n = 8) |             | Ex + AGE (n = 8) |              |
|---------------|-----------------|---------------|--------------|-------------|----------------------|-------------|------------------|--------------|
|               | Before          | After         | Before       | After       | Before               | After       | Before           | After        |
| HDL-C (mg/dl) | 55.8 ± 12.3     | 53.6 ± 10.5   | 67.3 ± 17.6  | 67.6 ± 14.9 | 62.5 ± 14.6          | 61.6 ± 29.3 | 55.8 ± 8.8       | 60.5 ± 13.1  |
| TG (mg/dl)    | 101.0 ± 29.9    | 138.6 ± 50.8* | 99.5 ± 39.6  | 81.3 ± 20.8 | 89.6 ± 41.1          | 77.5 ± 27.9 | 121.1 ± 47.4     | 101.6 ± 27.5 |

<sup>1)</sup> Values are means ± SD.

TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglyceride

\**P* < 0.05 vs before intervention**Table 3.** Effect of exercise and AGE intervention on markers of endothelial function in postmenopausal women<sup>1)</sup>

|                          | Placebo (n = 6) |             | AGEI (n = 8) |              | Ex + Placebo (n = 8) |              | Ex + AGE (n = 8) |              |
|--------------------------|-----------------|-------------|--------------|--------------|----------------------|--------------|------------------|--------------|
|                          | Before          | After       | Before       | After        | Before               | After        | Before           | After        |
| Nitrite/Nitrate (μmol/L) | 32.4 ± 18.9     | 27.2 ± 11.4 | 41.3 ± 15.6  | 22.5 ± 12.1  | 41.1.34.7            | 33.2 ± 7.6   | 44.0 ± 15.7      | 17.2 ± 15.1* |
| Endothelin-1 (pg/mL)     | 0.9 ± 0.4       | 1.4 ± 0.2   | 1.2 ± 0.4    | 1.2 ± 0.4    | 1.0 ± 0.2            | 1.5 ± 0.5    | 0.9 ± 0.4        | 1.3 ± 0.3*   |
| HR (beats/min)           | 64.1 ± 6.9      | 59.8 ± 7.0  | 69.1 ± 7.1   | 60.8 ± 6.5   | 65.7 ± 9.5           | 59.2 ± 5.5   | 63.8 ± 10.8      | 59.5 ± 6.1   |
| SBP (mmHg)               | 113.0 ± 8.0     | 109.8 ± 6.3 | 122.3 ± 20.9 | 125.3 ± 17.8 | 125.0 ± 14.0         | 120.1 ± 15.4 | 128.3 ± 13.6     | 126.1 ± 16.3 |
| DBP (mmHg)               | 71.1 ± 6.1      | 68.3 ± 5.1  | 69.1 ± 12.6  | 75.7 ± 11.8  | 65.7 ± 7.8           | 70.1 ± 11.5  | 63.8 ± 10.8      | 77.6 ± 14.0  |
| MAP (mmHg)               | 86.5 ± 4.0      | 83.5 ± 2.8* | 90.9 ± 15.0  | 90.7 ± 12.0  | 91.3 ± 10.0          | 87.3 ± 11.1  | 94.7 ± 13.1      | 93.1 ± 14.2  |
| PP (mmHg)                | 42.0 ± 6.5      | 39.5 ± 4.9* | 48.1 ± 9.7   | 49.1 ± 7.8   | 50.0 ± 11.6          | 49.7 ± 9.3   | 52.5 ± 4.8       | 52.1 ± 4.4   |

<sup>1)</sup> Values are means ± SD.

HR, heart rate at rest; SBP, carotid arterial systolic blood pressure; DBP, carotid arterial diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure

\**P* < 0.05 vs before intervention

statistically significant. LDL was significantly lower in the AGEI, Ex + Placebo, and Ex + AGE groups compared to the Placebo group (*P* < 0.01). MDA level was significantly lower in AGEI, Ex + Placebo (*P* < 0.01), and Ex + AGE groups (*P* < 0.05). Ex + Placebo group was significantly lower than among the groups (*P* < 0.05) (Fig.1). HDL-C was not different among the groups. TG was significantly increased in the Placebo group, but AGE or exercise suppressed this increase (Table 2).

### Endothelial function

Homocysteine was significantly decreased with intervention in AGEI, and Ex + AGE (*P* < 0.05) (Fig.1). Nitrate/nitrite level was significantly decreased in the Ex + AGE intervention (*P* < 0.05). However, ET-1 was significantly increased in the Ex + AGE group (*P* < 0.05). There were no significant differences between baseline and intervention in blood pressure, HR, SBP, or DBP at rest. MAP was slightly decreased in the Ex + Placebo group, however, it was not statistically significant. MAP and PP were significantly decreased in the Placebo group (*P* < 0.05). There were no statistically significant changes among the groups (Table 3).

### Discussion

The purpose of this study was to investigate the effects of a 12-weeks AGE regimen with regular exercise on CVD risk factors in healthy postmenopausal women by measuring body composition, lipid profiles, an oxidative stress marker, and vascular function. The major finding of this study demonstrates that AGE supplementation and/or exercise training results in beneficial effects on body weight, BMI, LDL-C, MDA, and

homocysteine, which are important CVD risk factors.

Menopause increases the risk of CVD because it affects metabolic parameters and endothelial dysfunction. These changes include increased fat mass, cholesterol level, and oxidative stress and, decreased vasodilatations, all of which can be controlled by exercise, nutrition, or a combination of the two treatment [16,22,32]. AGE is safe, and previous studies have demonstrated that it is more effective than garlic oil, powder, and raw garlic in relation to decreasing CVD risk factors [33,34]. In addition, AGE contains SAC, which is more easily absorbed than garlic power [35].

In the present study, the effect of AGE supplementation is primarily shown by decreased body weight and fat. Regular AGE supplementation significantly decreased body weight and fat compared to the Placebo group, and a similar effect was achieved with a 12-week exercise regimen. Although these findings demonstrate that AGE supplementation alone may inhibit increasing fat mass, we did not determine the mechanism. Furthermore, the combination of AGE and exercise synergistically promoted greater body weight loss than either treatment alone. Because obesity with high fat accumulation is a major risk factor for CVD, and weight loss decreases CVD risk, these results suggest that AGE intake potentially decreased CVD risk in postmenopausal women.

Many researchers have suggested that lifestyle modifications such as exercise and dietary changes were recommended as preventative measures for postmenopausal women [36-38]. In our study, 12 weeks of regular exercise training decreased body weight, LDL-C, and MDA in postmenopausal women. In agreement with our results, Figueroa *et al.* [31] demonstrated that exercise training improves metabolic parameters in postmenopausal women. However, we hypothesized that AGE supplementation

and exercise training would decrease CVD risk factors to a greater degree than either treatment alone. However, we did not observe an additive effect. These results indicate that the effects of AGE intake and regular exercise training may be independent. Importantly, decreased CVD risk may be achieved by AGE supplementation alone in postmenopausal women.

Nitrosative stress occurs when excessive reactive nitrogen species production in a system exceeds the organism's ability to neutralize and remove them. Nitric (NO) overproduction may alter protein structure via nitrosylation or lead to lipid peroxidation [39]. In our study, AGE supplement significantly reduced formation of nitrite/nitrate levels which is the end-product of NO.

Elevated plasma homocysteine is a risk factor for CVD that is independent of hypercholesterolemia [40]. Although there is controversy regarding the mechanism underlying homocysteine-induced CVD, there is a clear relationship between plasma homocysteine level and CVD risk; therefore, lowering plasma homocysteine is beneficial for reducing the incidence of CVD [41]. In agreement with animal experiments [42], AGE supplementation in postmenopausal women lowered plasma homocysteine, but exercise alone did not. We did not observe an additive effect in the group that received AGE and exercised. Our results indicate that AGE supplementation may be useful for postmenopausal women with elevated homocysteine levels.

It is well-known that ET-1 induces strong vasoconstriction activity in vascular smooth muscle cells [43]. Increased circulation levels of ET-1 in postmenopausal women have been previously reported [44]. In this study, we found that plasma ET-1 concentrations were only significantly increased in the Ex +AGE group. It is not clear why this group showed a significantly higher level of ET-1, but it may be related to the aging process or unidentified physiological change during menopause following AGE supplementation or exercise because the mean ET-1 value among the groups was similar.

The present study has several limitations. The sample size was small; eight participants in each group may not have been sufficient to obtain statistically significant results. A further study with a larger number of participants is necessary. Furthermore, a longer study period may yield more significant results.

In conclusion, we demonstrated that 12 weeks of AGE supplementation might provide independent benefits on reducing CVD risk factors for post menopausal women without hindering the well known beneficial effect of exercise.

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