Aged garlic extract supplementation modifies inflammation and immunity of adults with obesity: A randomized, double-blind, placebo-controlled clinical trial

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Background: Obesity is a serious global health issue and often results in low-grade systemic inflammation, increasing the risk for several chronic diseases. If obesity-induced inflammation could be reduced, fewer complications and co-morbidities might occur.

Objective: To investigate whether daily supplementation with aged garlic extract (AGE) could reduce chronic inflammation and improve immune function in adults with obesity.

Methods: Fifty-one healthy adults with obesity (mean age 45.6 ± 1.6 years, mean BMI 36.1 ± 0.9 kg/m²) were recruited to participate in a parallel, double-blind, placebo-controlled, randomized study. After being matched by BMI, participants were randomized into the AGE supplementation or placebo group. Participants were asked to take a divided daily dose of 3.6 g AGE or placebo, with food for 6 weeks. Blood lipid and inflammatory markers were assessed at baseline and after 6 weeks of supplementation. Additionally, peripheral blood mononuclear cells (PBMC) were isolated from whole blood and used to detect changes in immune cell populations and levels of cytokine secretion. A one-way ANCOVA was performed to evaluate differences between the two groups, controlling for respective baseline values.

Results: At the end of study, serum IL-6 (p = 0.04) and TNF-α (p = 0.05) of participants consuming AGE were significantly lower than those consuming the placebo capsules. PBMC flow cytometry results showed that participants from the AGE group had a higher proportion of γδ-T cells (p = 0.03) and a lower proportion of NKT cells (p = 0.02) in the total population of lymphocytes. There was no difference in percentage of NK cells between the two groups. A significant difference in blood LDL concentration was also observed (p = 0.05). Total cholesterol and non-HDL cholesterol tended to differ between participants from the AGE group and those from the placebo group, although values did not achieve statistical significance.

Conclusion: Six weeks of AGE consumption modulated immune cell distribution, prevented the increase of serum TNF-α and IL-6 concentrations and reduced blood LDL concentration in adults with obesity. AGE, taken consistently, may be beneficial in preventing the development of chronic diseases associated with low-grade inflammation in adults with obesity.

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1. Introduction

Obesity has become a serious health issue all over the world, especially in Western countries. In the United States, more than one-third of adults are obese [1]. Obesity often comes with an increased risk of systemic inflammation that stems from the immune system [2,3]. When the immune system is chronically...
activated, a barrage of free radicals, oxidative products and inflammatory cytokines assault the human body, damaging cells and organs. During obesity, adipocytes increase in both number and size, accumulating triglycerides and secreting a series of cytokines and adipokines, which leads to dysfunction of innate and adaptive immune cells. Natural killer (NK) cells represent the first line of defense by the innate immune system. Numbers of circulating NK cells have been shown to be reduced in patients with obesity [4,5]. However, when data from unhealthy patients with obesity was removed, NK cell counts in healthy patients with obesity was similar to that of lean participants [5,6]. Adaptive immune cells are also related to obesity. As obesity progresses, cytotoxic T cells are recruited into adipose tissue, leading to the depletion of adipose regulatory T cells and increased activation of T helper cells [7,8]. Gamma delta (γδ) T cells, a subset of T cells that express γδ TCRs, are innate lymphocytes that have been shown to respond to dietary bioactive components [9–12]. Their reduction in peripheral blood during obesity may contribute to obesity-induced inflammation and metabolic syndrome [13]. The molecular and cellular changes that occur during obesity increase the risk for metabolic diseases. A reduction of inflammation associated with obesity may diminish the development of complications, like cardiovascular disease.

Oxidative stress rate on NK cells is a predictor and a producer of inflammation. It stimulates the activation of mediator signaling molecules such as NF-κB, which can up-regulate the production of inflammatory cytokines [14]. Glutathione (GSH) is an endogenous tripeptide and is central to redox defense during oxidative stress, protecting host immune cells from free radicals. Daily consumption of dietary supplements that support GSH production could potentially be a method of counteracting the chronic inflammation associated with obesity.

A candidate for this type of supplementation would be aged garlic extract (AGE). After harvesting, the garlic is stored in an aqueous ethanol solution for up to 20 months. This converts the organosulfur compounds in garlic into milder and less odoriferous compounds, mainly S-allylcysteine (SAC). Numerous studies have demonstrated the health benefits of AGE. In human studies conducted by Reid et al. [15,16], AGE supplementation showed anti-hypertensive effects in patients with uncontrolled hypertension. Yeh et al. demonstrated that AGE was able to lower cholesterol in both human and rats [17]. AGE may also have neuroprotective effects. AGE delayed the appearance of infarct area in a cerebral ischemia rat model [18] and improved spinal cord injury outcomes in rats [19]. Additional health benefits of AGE may include the prevention of cancer and diabetes. AGE inhibited the growth of tumor cells in both in vitro and animal studies [20,21]. This preventive effect was also seen in humans. In a randomized, double-blind human study, AGE supplementation inhibited the growth of new adenomas and decreased the total size of adenomas in patients who were diagnosed as having colorectal adenomas [22]. Thomson et al. studied the effect of different doses of AGE on diabetes control in rats with diabetes induced by streptozotocin, and observed a dose-dependent improvement in diabetes control [23]. In our previous study, we found that AGE modulated human immunity [24,25]. In that study, 120 healthy, non-obese adults were randomized to consume AGE (2.56 g) or placebo capsules, daily, for 90 days. γδ-T and NK cells from participants who consumed AGE showed greater proliferation after stimulation, than those of participants who consumed the placebo. In addition, NK cells of those consuming AGE had more MHC2D, a marker of cell activation which plays an important role in NK cell cytotoxicity [26], expressed on their surface. In the same study, a decrease in the secretion of inflammatory cytokines from cultured peripheral blood mononuclear cells (PBMC) was observed. These two results suggest that AGE supplementation improved the natural killing capability of immune cells, while fewer inflammatory cytokines were being secreted. The purpose of the current study was to extend our knowledge regarding AGE supplementation and its potential benefit of supporting immunity, by studying adults with obesity, many of whom also have concurrent systemic inflammation. We hypothesized that AGE supplementation would reduce obesity-associated chronic inflammation in healthy adults with obesity through a modulation of their immune system.

In order to examine this, we looked at the percentage of specific populations of lymphocytes in the total PBMC population, as well as concentrations of inflammatory cytokines in the serum, before and after the 6-week AGE intervention. We also evaluated the effect of daily AGE supplementation on blood lipid levels.

2. Materials and methods

2.1. Study design and participants

A total of 175 healthy adults with obesity who responded to public recruitment messages, were assessed for eligibility to participate in this double-blind, randomized, placebo-controlled clinical trial between July of 2014 and September of 2015. Of the 73 adults that met the inclusion criteria, 55 chose to enroll (Fig.1). All participant visits were conducted in the Clinical Nutrition Laboratory of the Food Science and Human Nutrition Building on University of Florida campus. Informed consent was obtained from all participants by trained personnel. This study was approved by the Institutional Review Board at the University of Florida and registered at ClinicalTrials.gov with the identifier code NCT01959646.

The inclusion criteria for the study were: 25–65 years of age, waist circumference (WC) > 88 cm for women or > 102 cm for men, body mass index (BMI) > 30 kg/m², be willing to discontinue other dietary supplements, and not be taking any medication for cardiovascular disease. In addition, those with blood pressure greater than 130/85 mmHg, metabolic syndrome, diabetes, arthritis, severe allergies or other immune disorders were excluded from participating. Metabolic syndrome is defined as the presence of three or more of the following risk factors: WC > 88 cm for women or > 102 cm for men, blood pressure ≥130/85 mmHg, triglycerides ≥150 mg/dL, HDL cholesterol ≤40 mg/dL for men or ≤50 mg/dL for women, and fasting glucose ≥100 mg/dL [27].

2.2. Sample size determination

To determine the minimum number of participants needed to detect a difference between the AGE and placebo groups in our primary outcome, γδ-T cell numbers, and secondary outcomes, inflammatory markers, a power analysis was conducted. With an alpha level of 0.05 and a power of 0.80, based on previous data where the average percentage of γδ-T cells in peripheral blood of participants in the placebo group was 4.7% ± 2.7 and those in a previous intervention group averaged 9.9% ± 5.3, it is indicated that 15 individuals per group would be needed. To determine the number of participants needed per group to see a statistical reduction in C-reactive protein (CRP) levels from an average value of 3.0 mg/dL, we applied interquartile differences of 1.14 to 1.05 mg/dL, and found we would need 32 participants.

2.3. Study procedures

To determine eligibility, height, weight, blood pressure and a finger-prick blood sample were obtained. Eligible participants with similar BMI (difference of less than 3 kg/m²) were matched as pairs and returned in the fasting state to the Clinical Nutrition Laboratory for a randomization visit. To ensure blinding of investigators and
participants, capsules were supplied by Wakunaga of America Co., Ltd. (Mission Viejo, CA, USA), labeled as either A or B, and contained either AGE (0.6 g/capsule) or a placebo. A blind drawing was done to assign one of each subject pair to group A, while the other was assigned to group B. They were then given numbers from a randomization table provided by Wakunaga of America Co., Ltd. Both participants and investigators were blinded to the intervention assignments. Venous blood was drawn into heparinized vacuum tubes for PBMC isolation, and into serum collection tubes. Participants were given bottles of their assigned intervention, instructed to consume three capsules with food, twice a day for 6 weeks (3.6 g AGE/day) and encouraged to continue their current dietary and exercise routines. After 3 weeks, participants returned bottles containing unused capsules and were given their second 3-week supply. After 6 weeks, participants again returned bottles containing unconsumed capsules for compliance assessment, underwent a second fasting blood draw, and completed a final questionnaire that asked about side effects experienced, consumption of other dietary supplements and medications, any significant changes in diet or exercise, and which treatment they thought they had consumed.

2.4. Anthropometric and biochemical measurements

Height, weight, blood pressure, WC and sagittal abdominal diameter (SAD) were measured at the beginning and end of the intervention period. Whole blood was used to determine biochemical markers, including total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), non-high density lipoprotein cholesterol (non-HDL), and glucose (GLU), using the Cholestech LDX® System (Alere™ Inc., Waltham, MA, USA) at the same time points.

2.5. Separation and culture of PBMC

To isolate PBMC, whole blood was layered on Lympholyte H® Cell Separation Media (Cedarlane Laboratories Ltd., Burlington, NC, USA), as previously described [10]. Freshly isolated PBMC (1 × 10⁶) were used to determine different types of immune cells. Another 1 × 10⁶ PBMC were cultured in wells of a 24-well plate in RPMI-1640 (Cellgro; Mediatech, Herndon, VA, USA) complete medium (100 U/ml penicillin; 100 µg/ml streptomycin; 2 mM l-glutamine; 25 mM HEPES), containing IL-2 (1 ng/mL), IL-15 (1 ng/mL), β-Mercaptoethanol (50 mM) and PHA-L (10 µg/mL) for stimulation, in a humidified, 5% CO₂ environment at 37 °C. After 24 h, cell culture medium was collected for determination of inflammatory cytokine concentrations; cells were collected for measuring cell proliferation.

2.6. Immune cell distribution within the total PBMC population

Flow cytometry was used to detect different types of immune cells. Cell surface markers were used to identify γδ-T cells (FITC-α human CD3, Alexa Fluor® 647-α human γδ-TCR), and NK and NKT cells (FITC-α human CD3, PE-α human CD56). All antibodies were

![Flow diagram of participants assessed for eligibility, enrolled in the AGE study, randomized to different treatments and included in analyses.](Fig. 1. Overall flow of participants assessed for eligibility, enrolled in the AGE study, randomized to different treatments and included in analyses.)
obtained from eBioscience, San Diego, CA, USA. Cells were incubated with antibodies at 4 °C in the dark for 30 min, washed, centrifuged, fixed with 1% paraformaldehyde, and run through a BD Accuri™ C6 Plus flow cytometer (Becton Dickinson, San Jose, CA, USA) within 48 h. Data were analyzed using FlowJo (version 10.1) analysis software (FlowJo, LLC., Ashland, OR, USA). Results are presented as a percentage of the total lymphocyte population.

2.7. Total glutathione and C-reactive protein level determination

Total intracellular glutathione was measured colorimetrically from 1 × 10⁷ PBMC homogenates using a commercial glutathione assay kit (Cayman, Ann Arbor, MI, USA) following kit directions.

The CRP concentration in the serum was determined using a human C-Reactive Protein/CRP Quantikine® ELISA Kit (R&D System Inc., Minneapolis, MN, USA), according to the manufacturer’s instructions.

2.8. Inflammatory cytokine determination

Concentrations of IL-6, TNF-α, leptin and total adiponectin in the serum and IL-6, TNF-α, MIP-1α and IL-10 in supernatant cell culture medium were determined using human cytokine multiplex immunoassay kits (Milliplex® Map Kit, EMD Millipore Corp., Billerica, MA, USA). The assays were performed following manufacturer’s directions.

2.9. Statistical analysis

All statistical analyses were performed using SAS JMP, v10 (SAS Institute, Cary, NC, USA). Descriptive statistics were used to show the demographic and anthropometric characteristics of participants. A one-way analysis of covariance (ANCOVA) was conducted to analyze the differences in all parameters between the two groups at the end of the study, while controlling for respective baseline values. Categorical data was analyzed by using Chi-squared test. There were three participants with compliance of less than 80%, so data collected from those participants were not used for analysis. Two additional participants reported illnesses at the post-intervention blood draw. Statistical analyses were performed both with and without those two participants and, as no differences were observed, those data were included in all analyses. All data are presented as the mean ± SEM, with p < 0.05 considered as significant.

3. Results

3.1. Baseline demographics and additional participant information

We examined subject demographics for differences between those randomized to consume AGE or the placebo and found that the groups were comparable in age, gender distribution, anthropometric parameters and blood biochemical parameters, except for baseline TG levels (Table 1). After 6 weeks of intervention, no changes were observed in blood pressure, body weight and other anthropometric measures. Self-reported side effects, illnesses and related behaviors of participants throughout the study are found in Table S1.

3.2. Intervention compliance

Based on capsule counts, average compliance levels were 93.4% in the placebo and 92.2% in the AGE groups (Fig. 2A). We determined cellular GSH concentrations as an indirect measure of compliance. Although changes from baseline of total PBMC glutathione concentrations were not significantly different between the two groups (p = 0.06), the level tended to increase in the AGE group (Fig. 2B). In our previous study, AGE supplementation increased intracellular GSH concentrations in PBMC of healthy adults [25]. The lack of the significance in the current study may be due differences between the populations studied, since all forms of glutathione are depleted in adults [28]. In addition, the difference in the length of study may be another factor. The current study lasted for 6 weeks, compared to 4 months in the previous study.

3.3. Immune cell distribution

Table 2 shows the proportions of γδ-T, NK T and NK cells in the total PBMC population. At the end of the intervention period, the percentage of γδ-T cells in the AGE group (p = 0.03) was

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographic and clinical characteristics of participants at baseline and completion of the study.</th>
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<tbody>
<tr>
<td></td>
<td>Total</td>
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<td>Demographics</td>
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<tr>
<td>n</td>
<td>48</td>
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<tr>
<td>Age (y)</td>
<td>44.0 ± 11.5</td>
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<td>Gender (n, %)</td>
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<tr>
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<td>Female</td>
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<tr>
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<td>DBP (mmHg)</td>
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<tr>
<td>Baseline</td>
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<td>TG (mg/dL)</td>
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<td>HDL (mg/dL)</td>
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<td>Non-HDL (mg/dL)</td>
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<td>LDL (mg/dL)</td>
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<td>GLU (mg/dL)</td>
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<tr>
<td>Week 6</td>
<td>98.0 ± 29.2</td>
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</table>

Data are presented as the mean ± SD. A one-way ANCOVA was performed to test for differences in anthropometric and clinical markers between the two groups, controlling for respective baseline measures. SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; WC: waist circumference; SAD: sagittal abdominal diameter; TC: total cholesterol; TG: triglycerides; HDL: high density lipoprotein cholesterol; non-HDL: non-high density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol; GLU: glucose.

* Indicates statistically significant between-group difference, p < 0.05.
Data are presented as the mean ± SEM. NK: natural killer.

3.4. Markers of inflammation

Several serum markers of inflammation were examined before and after the intervention (Table 3). In response to the intervention, significant differences were seen in IL-6 and TNF-α levels between the two groups. After the intervention, participants consuming AGE had lower serum IL-6 (p = 0.04) concentration and lower serum TNF-α concentration (p = 0.05) on controlling for respective baseline measures.

No significant differences in CRP, leptin, and total adiponectin were observed between the two groups in response to the intervention. The average concentration of CRP in the serum before the intervention was higher than normal in both the placebo (5.7 mg/dL) and AGE (4.4 mg/dL) groups. After the intervention, the levels of CRP were very similar to baseline. No significant differences in CRP, leptin, and total adiponectin were observed between the two groups.

While there was an observed decrease of both TC and non-HDL in response to the intervention differed between the AGE and placebo groups. After six week of supplementation, blood LDL concentration in the AGE group, the average LDL concentration was 118.0 ± 28.4 which was higher than that in the placebo group (107.7 ± 31.3). While there was an observed decrease of both TC and non-HDL in the AGE group, differences between the AGE and control groups were not significant for either parameter. Although TG concentrations were different at baseline between the two groups, there were no differences in TG concentration between the two groups during the intervention period. There were also no significant differences in the HDL and GLU levels between the two groups. Data are showed in Table 1.

3.5. Clinical parameters

After six week of supplementation, blood LDL concentration in response to the intervention differed between the AGE and placebo groups (p = 0.05), controlling for baseline LDL concentrations. In the AGE group, the average LDL concentration was 118.0 ± 28.4 which was higher than that in the placebo group (107.7 ± 31.3). While there was an observed decrease of both TC and non-HDL in the AGE group, differences between the AGE and control groups were not significant for either parameter. Although TG concentrations were different at baseline between the two groups, there were no differences in TG concentration between the two groups during the intervention period. There were also no significant differences in the HDL and GLU levels between the two groups. Data are showed in Table 1.
4. Discussion

In this 6-week, double-blind, randomized, placebo-controlled clinical study, we determined the effects of AGE supplementation on immune cells and markers of inflammation. In adults with obesity, we found that 6 weeks of AGE supplementation was able to modulate immune cell distribution and prevent the increase of serum concentrations of IL-6 and TNF-α, factors that play important roles in the development of obesity-induced inflammation. Moreover, blood LDL levels, which often increase with obesity and are a major health concern [29], were significantly lower after AGE supplementation.

4.1. AGE and inflammatory mediators during obesity-induced inflammation

Adipocytes, the major cell type present in adipose tissue, can secrete IL-6, leptin, and adiponectin. In obesity, adipocytes tend to increase in both number and size, which results in excess adipokine secretion and a pro-inflammatory environment in the adipose tissue. It is well established that levels of inflammatory mediators in the serum increase in obesity, and are positively correlated with BMI and WC [30–32]. Confirming these findings, we found that serum CRP, IL-6, TNF-α and leptin concentrations were all elevated at baseline, an indication of inflammation in the obese participants in our study. After the intervention, we noted that those consuming AGE had lower serum IL-6 and TNF-α concentrations after the six-week intervention, suggesting that AGE supplementation might help to prevent the progression of inflammation. We did not find any changes in serum CRP or leptin levels. Very few human studies have assessed the effect that consumption of AGE or garlic-associated compounds might have on CRP. A study conducted by Zeb et al. [33] showed that the consumption of a capsule containing AGE and Coenzyme Q10 for 12 months significantly decreased CRP levels compared with a placebo. Since the capsule contained both AGE and Coenzyme Q10, the authors were unable to attribute the anti-inflammatory effect they saw to either one of the compounds. In addition, the participants in that study were all male firefighters, a very distinct population. In another study, garlic powder (2.1 g/d) was consumed by overweight participants for three months and, as we found, there were no significant differences in serum CRP levels between the groups after the intervention [34]. The effect of AGE consumption on leptin has only been studied in animals thus far. Perez-Torres et al. showed that rats with metabolic syndrome had elevated serum leptin concentrations and that AGE returned them to control levels [35]. However, this benefit was not seen in our study. It may be due to the differences in metabolism between humans and rats.

In the obese, concentrations of anti-inflammatory mediators are reduced and are negatively correlated with BMI [36]. One of those anti-inflammatory mediators, adiponectin, is secreted by adipose tissue. Ryo et al. [37] showed that adiponectin levels are negatively associated with the clinical phenotype of metabolic syndrome. In a study conducted by Gómez-Arbelaez et al. [38], AGE supplementation improved adiponectin levels in obese adults with metabolic syndrome. In our study, where having metabolic syndrome was exclusionary, we saw no change in serum adiponectin levels in either group after the 6-week intervention. All of our participants had relatively high concentrations of adiponectin prior to the intervention, with average baseline concentrations of 14.2 μg/mL, more than double the 5.9 μg/mL in the Gómez-Arbelaez study. The differences in metabolic health and initial adiponectin concentrations of participants in our study and in that of Gomez-Arbelaez, may explain the variation in the adiponectin response to AGE.

Cytokines secreted by cultured PBMCs were also assessed, but found not to differ between treatments. The reason is not clear, however variability among participants was apparent.

4.2. AGE and immune cell populations during obesity-induced inflammation

Similar to changes in inflammatory mediators, alterations in immune cell population numbers were also observed. Obesity has a detrimental impact on γδ-T cell populations because of the low-grade chronic inflammation it produces. Human obesity is associated with a reduction of γδ-T cells in the peripheral blood, and the number is negatively correlated to the severity of obesity [13]. In contrast, results from an animal study showed an increase in the number of γδ-T cells in adipose tissue [39]. The decrease in the number of γδ-T cells in peripheral blood may be due to their infiltration into adipose tissue. In fact, we also found a low baseline percentage of γδ-T cells in the blood of the healthy obese adults enrolled in our study. While γδ-T cells increase in adipose tissue, NK cells are diminished from it [40]. CD3+CD56+ NK cells are highly enriched in liver and adipose tissue, but rare in peripheral blood, around 2% [41]. At the start of our intervention NK cells comprised a much higher proportion (6.7%) of that lymphocyte population, perhaps as a result of the migration of those cells from adipose tissue into the circulation. After supplementation with AGE, we saw an increase in the percentage of γδ-T cells and a decrease in the percentage of NK cells in the circulating blood of our obese participants, an indication that AGE supplementation had modulated their immunity. Since the participants in our study were healthy obese adults, we did not note any changes in NK cell numbers, consistent with results from previous studies [5,6].

4.3. AGE and risk factors of cardiovascular disease during obesity-induced inflammation

The cardiovascular benefits of AGE supplementation, including the anti-hypertensive and cholesterol-lowering effects, have been illustrated in numerous studies. Ried et al. demonstrated that AGE was effective in lowering blood pressure in patients with uncontrolled hypertension [15]. In that study, participants were asked to consume 0.96 g/day of AGE for 12 weeks, or a placebo, and blood pressure was measured at baseline, 4, 8 and 12 weeks. Participants with uncontrolled hypertension in the AGE group experienced a decrease in systolic blood pressure throughout the 12-week intervention period. In our study, all participants started with blood pressure levels of less than 130/85 mmHg, which perhaps explains why we did not note any anti-hypertensive effects.

As early as 1996, Steiner et al. showed that daily consumption of 7.2 g of AGE for 6 months had beneficial effects on the lipid profile of moderately hypercholesterolemic participants, with decreases in TC (7.0%) and LDL (4.6%) levels [42]. Yeh et al. demonstrated a cholesterol-lowering effect of AGE using both human and animal studies [17]. In the human study, 36 hypercholesterolemic participants, with an average BMI of 25.5 kg/m², were asked to consume 7.2 g of AGE per day for 5 months. After the intervention, the level of TC had decreased by 7% and the level of LDL cholesterol had decreased by 10%. Similar results were observed in their animal study, where AGE supplementation lowered total cholesterol concentration by 15% LDL levels in our participants that consumed AGE decreased by 5.2%. There was also a trend for TC and non-HDL concentrations to decrease, indicating that AGE did improve the lipid profile of adults with obesity. Although the LDL decrease that we observed was not as pronounced as that of earlier studies, this could be due to our shorter intervention period.
4.4. Strengths and limitations

This study has several strengths. To our knowledge, this is the first report that evaluates the effect of AGE supplementation on immune cell populations in adults with obesity-induced inflammation. As obesity affects more than 600 million adults worldwide [43], identifying successful interventions to moderate the negative effects of obesity, like systemic inflammation and associated chronic diseases such as CVD, are imperative. Here, we not only investigated the response of immune cell populations, but also inflammatory mediators. Participants were asked to maintain their usual diet and activity patterns, so body weight remained unchanged throughout the intervention period and was not a confounding factor. However, the variation in AGE dose and participant characteristics does not allow for direct comparisons of current results with those from prior studies. Participants in our intervention group consumed 3.6 g/day of AGE for 6 weeks and were obese, without metabolic syndrome. Other investigations discussed here provided doses ranging from 0.96 g/day to adults with uncontrolled hypertension to 7.2 g/day for adults with hypercholesterolemia. Furthermore, the length of the intervention periods differs significantly among the studies. We were not able to evaluate the long-term effects of AGE supplementation on inflammation and immune function in adults with obesity, as our intervention lasted only 6 weeks. The other studies had AGE intervention periods that ranged from 12 weeks to 6 months. Given these limitations and our findings, we believe that additional immunology studies with longer intervention periods are warranted to determine the long-term benefits of AGE consumption on the immune health of adults with obesity.

In summary, the changes in immune cells and inflammatory mediators that we found show that the consumption of AGE can produce an anti-inflammatory effect in adults with obesity. However, the mechanism by which AGE exerts its effect on obesity-induced inflammation is still not clear. One potential mechanism may involve hydrogen sulfide (H2S), a recently discovered second messenger shown to prevent the activation of the NF-kB signaling pathway, which consequently attenuates the production of pro-inflammatory cytokines [44,45]. The organosulfur compounds in aged garlic extract, such as SAC, have been suggested as H2S mediators by increasing its endogenous production [46,47], which may drive the suppression of obesity-induced inflammation. We suggest that future studies focus on determining the mechanism by which AGE affects obesity-induced inflammation, to increase the understanding of the potential health benefits of AGE consumption.

Conflict of interest statement

The authors all declare that they have no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Statement of authorship

SSP and AEM designed the research. CX, CR, BJE, CAR and AO conducted the research and collected the data. CX and SSP performed the statistical analysis; CX, AEM, CAR and SSP co-authored the manuscript and SSP is responsible for the final content.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.clnesp.2017.11.010.

References


