A double-blind crossover study in moderately hypercholesterolemic men that compared the effect of aged garlic extract and placebo administration on blood lipids\textsuperscript{1,2}

Manfred Steiner, A Hakim Khan, Don Holbert, and Robert I-San Lin

ABSTRACT A double-blind crossover study comparing the effect of aged garlic extract with a placebo on blood lipids was performed in a group of 41 moderately hypercholesterolemic men (cholesterol concentrations 5.7–7.5 mmol/L (220–290 mg/dL)]. After a 4-wk baseline period, during which the subjects were advised to adhere to a National Cholesterol Education Program Step I diet, they were started on 7.2 g aged garlic extract per day or an equivalent amount of placebo as a dietary supplement for a period of 6 mo, then switched to the other supplement for an additional 4 mo. Blood lipids, blood counts, thyroid and liver function measures, body weight, and blood pressure were followed over the entire study period. The major findings were a maximal reduction in total serum cholesterol of 6.1\% or 7.0\% in comparison with the average concentration during the placebo administration or baseline evaluation period, respectively. Low-density-lipoprotein cholesterol was also decreased by aged garlic extract, 4\% when compared with average baseline values and 4.6\% in comparison with placebo period concentrations. In addition, there was a 5.5\% decrease in systolic blood pressure and a modest reduction of diastolic blood pressure in response to aged garlic extract. We conclude that dietary supplementation with aged garlic extract has beneficial effects on the lipid profile and blood pressure of moderately hypercholesterolemic subjects. Am J Clin Nutr 1996; 64:866–70.

INTRODUCTION An increased serum cholesterol concentration is an important risk factor for the development of cardiovascular and possibly cerebrovascular disease. Similarly, enhanced platelet response to activating agents represents a distinct risk for thromboembolic events in the arterial circulation. Reduction of these and other risk factors through dietary modification, behavioral changes, and medicinal intervention has already substantially decreased the incidence and mortality from coronary and cerebrovascular disease. Supplementation of the diet with certain biofactors may further reduce such risk factors (1, 2). Garlic belongs to a group of dietary supplements that may lessen the incidence of cardio- and cerebrovascular disease by reducing cholesterol concentrations and decreasing platelet responsiveness to activating agents.

Several studies were published over the past two decades that ascribe a hypocholesterolemic action to garlic preparations (3–14). Although there are substantial differences in the composition of garlic preparations, many of those currently on the market as well as fresh garlic have been reported to decrease total cholesterol significantly. A careful and critical review of the data from authentic scientific studies led one of us to estimate that dietary supplementation with \(\approx 10-15\) g cooked garlic/d or equivalent amounts of garlic oil or aged garlic extract (AGE) can lower serum cholesterol by \(\approx 5-8\%\) in the majority of hypercholesterolemic persons (1). Some investigators, however, have found no cholesterol-lowering effect of garlic (15). There is a lack of well-controlled intervention trials that can validate such an estimation, especially trials in which vital signs, body weight, blood chemistry results, and liver functions of the study subjects are carefully monitored. Changes in some of these variables can have substantial effects on the serum cholesterol concentration. For this reason we undertook a study in which all of these variables were monitored during an 11-mo study.

We performed a double-blind crossover study in a group of moderately hypercholesterolemic men who were given AGE over a period of 4–6 mo. We measured serum lipids, including total cholesterol, low-density-lipoprotein (LDL) and high-density-lipoprotein (HDL) cholesterol, and triacylglycerols, and monitored their blood pressure.

SUBJECTS AND METHODS

Study population Fifty-six men aged 32–68 y were enrolled in the double-blind crossover study. A prerequisite for enrollment was normal results from a physical examination and a total cholesterol concentration between 5.7 and 7.5 mmol/L (220 and 290 mg/dL). All participants were advised to follow the National Cholesterol Education Program guidelines.\textsuperscript{1,2}

\textsuperscript{1} From the Memorial Hospital of Rhode Island, Pawtucket, and Brown University School of Medicine, Providence, RI; East Carolina University, Greenville, NC; and Nutrition International Co, Irvine, CA.

\textsuperscript{2} Address reprint requests to M Steiner, East Carolina University, School of Medicine, Department of Medicine, Hematology/Oncology, Brody Building 3E-127, Greenville, NC 27858-4354. E-mail: Steiner@brody.med.ecu.edu.

Received June 20, 1996. Accepted for publication July 11, 1996.

The garlic education program step 1 diet (16) for the length of the study. During a 4-wk period baseline values of lipid profiles (total, HDL, and LDL cholesterol, and triacylglycerols) were analyzed at weekly intervals, and the subjects were then randomly assigned to one of two study arms to receive either nine placebo or nine AGE capsules (Wakunaga of America Co, Mission Viejo, CA), which were taken in three divided doses with meals daily as a dietary supplement. Both types of capsules were identical in appearance and were custom-made for this study. Each garlic capsule contained 800 mg AGE powder whereas the placebo capsule contained 600 mg corn starch, 99.5 mg microcrystalline cellulose, 0.5 mg caramel, and 3.5 mg magnesium stearate. The first intervention period began in week 5 and lasted for 180 d, during which time lipids were analyzed six times. After that, individuals were changed to the supplement they did not receive during the first intervention period and continued the study for a total of 120 d, during which period blood lipids were measured five times. Measurements were performed by MetPath (Corning Clinical Laboratory, New Britain, CT). Cholesterol was measured enzymatically by using a cholesterol esterase test with a reproducibility of 2%. LDL-cholesterol values were calculated by using the Friedewald equation (17). Hemoglobin, hematocrit, and white blood cell values as well as serum creatinine and thyroid and liver functions were checked four times, once during the baseline study period, twice during the first intervention period, and once at the end of the second intervention period. Body weight was checked once during the baseline period, six times during the first intervention period, and five times during the second intervention period. Blood pressures were monitored by manual measurement with a sphygmomanometer.

This investigation was reviewed and approved by the Human Studies Review Board of the institution where it was conducted. All volunteers signed an informed consent form before enrollment in the study.

Statistical evaluation

All data are presented as means ± SDs at each of the 15 sampling points. The latter are described throughout the paper as −4 to −1 for the four weekly baseline evaluations and as 1–11 for the 11 intervention-period measurements that were performed at 30-d intervals except for the first, which came 10 d after the intervention trial began. Each response variable was analyzed according to the methods described for crossover designs by Cochran and Cox (18). Analyses were conducted by using the general linear models procedure and plotting procedures from SAS software (19). Fisher's least-significant-difference test (18) was used to compare treatment regimens. P values < 0.05 were considered significant. We evaluated all data comparing the effect of dietary supplements within each study arm and also across the two study arms. Arm 1 study subjects began their intervention trial with AGE, whereas arm 2 study subjects started with placebos.

RESULTS

We screened 75 men, most of whom were former participants in the Pawtucket Heart Health Study and known to have moderately high serum cholesterol concentrations. Fifty-two met our entrance criteria and were enrolled. Of these individuals, 11 dropped out during the first or second intervention period for a variety of reasons that included allergy to the coating material of the capsules, various gastrointestinal complaints, difficulties in taking the required number of capsules, and perception of unusual body odor. Forty-one men completed both arms of the study. There were no significant differences in any of the blood count or blood chemistry values in the study subjects for the placebo and AGE intervention periods nor between the latter and baseline. Similarly, thyroid screening test results were not affected by the study supplements. Note that animal experiments have shown that thyroid uptake of [31]I becomes abnormal after injection of high doses of allyl sulfides (20). However, the AGE used in this study contains only trace amounts of these thiaoallyl compounds. Body weights of those who started with the garlic supplement were 85.8 ± 13.0, 86.3 ± 10.1, and 85.7 ± 10.6 kg, respectively, for the baseline, first, and second intervention periods. Study participants starting with the placebo supplement weighed 85.8 ± 16.3, 86.8 ± 17.0, and 86.0 ± 16.8 kg for the baseline, first, and second intervention periods.

Systolic blood pressures (Figure 1A and B) showed significant reductions with AGE supplementation not only compared with placebo, but also when compared with baseline. A

FIGURE 1. Systolic blood pressure readings in study subjects who began their intervention trial with aged garlic extract (AGE) (study arm 1; A) or with placebo (study arm 2; B) supplements. Within-study arm comparisons: AGE compared with baseline, P = 0.0001 (both study arms); placebo compared with AGE, P = 0.003 and 0.0001; and placebo compared with baseline, P = 0.0045 and 0.0003 for study arms 1 and 2, respectively. Across-study arm comparisons: AGE compared with placebo, P = 0.0001; AGE compared with baseline, P = 0.0001; and placebo compared with baseline, P = 0.0002.
baseline but also with placebo, and irrespective of whether such comparisons were made within or across each study arm. Significant reductions were also noted between baseline and placebo intervention periods. At maximal reduction during AGE administration, systolic blood pressures were 5.5% lower than the average pressure during placebo supplementation. Diastolic blood pressures (Figure 2) were also significantly reduced during the AGE compared with the placebo intervention period, but when differences within each study arm were evaluated separately, it became apparent that study subjects taking AGE supplements during the first intervention period showed no significant change in values from either baseline or the placebo period.

The lipid profiles of study participants are shown in Figures 3–6. Irrespective of whether the men started the first intervention period with AGE or placebo and whether comparisons were made within or across study arms, total serum cholesterol was significantly reduced with AGE administration (Figure 3, A and B). At the time of maximal reduction, total cholesterol concentrations of subjects averaged in both study arms were 6.1% below the mean concentrations of all study participants with placebo and 7.0% below their baseline values. LDL-cholesterol values for individuals taking AGE were also reduced compared with those taking placebo (Figure 4). Within-study arm analyses showed significant reductions after AGE in comparison with the placebo, but only in subjects starting their dietary intervention with placebo supplements did the difference between baseline and AGE become significant. The maximal reduction of LDL cholesterol was 4% when the average of both study arms was compared with baseline values and 4.6% compared with the average placebo concentrations. Lipid-lowering became apparent within one to two sampling periods, ie, 10–40 d, and reached a nadir = 3 mo after subjects switched to AGE. HDL-cholesterol and triacylglycerol concentrations were not significantly changed by either of the two dietary supplements (Figures 5 and 6, respectively). Comparisons of the ratios of HDL to total cholesterol across study arms showed a significant increase (P = 0.0145) after AGE compared with placebo supplementation (0.19 ± 0.04 compared with 0.18 ± 0.04).

Compliance, which was measured by pill counting, was somewhat better in participants who started with placebos than in those starting with AGE. Among the latter, 8 of 17 subjects showed pill counts that were within 5% of the expected value. More than one-half of the participants in this group had pill counts that were ≥ 20% off the expected value. On the other hand, 8 of 24 subjects starting with placebos had pill counts that were ≥ 20% off the expected value. A variety of reasons were given for poor compliance, the most common one was forgetting to take lunch-time doses of the supplement.

**DISCUSSION**

Many supplementation trials with garlic have been performed over the past 15 y. Recently, two meta-analyses evaluated the published trials on the basis of objective criteria for comparison (21, 22). The authors of both studies concluded that garlic supplements produced a reduction in cholesterol
concentrations ranging from 9% to 12%. We believe that this range is too high, above the 5–8% reduction in total serum cholesterol estimated by Lin (1) in a critical analysis of the existing data. Triacylglycerol concentrations also decreased during garlic therapy by ≈13% in eight trials, all of which used garlic powder preparations. HDL cholesterol was not significantly lowered however. In one study of 42 healthy adults who had cholesterol concentrations ≥5.7 mmol/L (≥220 mg/dL), LDL cholesterol was reduced 11% by garlic compared with 3% by placebo treatment, but there were no changes in HDL cholesterol or triacylglycerols (14). The reductions in total cholesterol obtained by nonpowder preparations of garlic were in general somewhat higher than those of powder preparations (22).

From the standpoint of acceptability of garlic as a dietary supplement, its composition and method of preparation are of great importance. Most of the garlic powder tablets contain substantial amounts of odorous compounds, e.g., diallyl sulfides or polysulfides responsible for the characteristic aroma of crushed and stale garlic. On the other hand, steam-distilled garlic oils as well as oil-macerated garlic contain only polysulfides and other volatile thioalyls. During steam distillation much of the alliin is converted to diallyl sulfides. Many of the thioallyl compounds are quite unstable. Allicin, the oxide of diallyl disulfide, is only a transient constituent of garlic preparations and under physiologic conditions does not pass through the intestine and enter the blood stream. Injected allicin is rapidly destroyed in the blood (1). Similarly, other oxygenated thioalyls, such as ajoene, are also unstable and have low bioavailability. The preparation used in this study has fewer unstable thioalyls but is relatively rich in S-allylcysteine with smaller amounts of S-allymercaptocysteine. These compounds are the result of an aging process that leads to the reduction of alliin (S-allylcysteine sulfoxide). Aged garlic preparations are far less odorous than fresh garlic extracts or garlic powders. Nevertheless, in high concentrations some odor is associated with AGE capsules although it is somewhat different from the typical garlic odor. In our study most subjects (~70%) were able to clearly identify whether they were taking garlic supplements or placebos. This led to a relatively high rate of noncompliance.

Dietary supplementation with AGE did not change blood counts or chemistry test results, nor did it affect thyroid function. Garlic supplementation has been reported to produce a modest reduction in blood pressure (5, 6, 9). Our studies confirm this finding.

Consumption of AGE reduced total cholesterol and LDL cholesterol, which reached a maximum effect around the third month after AGE supplementation started. There was not a significant difference in HDL cholesterol or triacylglycerols between placebo and AGE periods nor between baseline and the intervention periods. The ratio of HDL to total cholesterol was significantly higher when subjects were consuming AGE compared with placebo. Although, in theory, this ratio should be a good predictor of risk for coronary artery disease, the inability to provide accurate HDL-cholesterol measurements has caused national expert panels to caution against use of this ratio (23). Even though our study showed a very significant AGE-induced reduction of total and LDL cholesterol, one wonders what effect might have been observed in individuals with better compliance. Whether a smaller dose of the aged garlic extract could have produced greater compliance and in turn a comparable reduction in cholesterol also remains unknown. However, it is apparent that removal of most of the
odor-producing substances from the garlic preparation did not completely eliminate objections to the ingestion of garlic extracts in high doses by some of some participants. Although comparisons between this and other garlic studies are difficult to make in view of the differences in the garlic preparations administered, nevertheless, our results are comparable with the estimated hypocholesterolemic effects of garlic supplements (1). All of our study participants were men with moderately elevated cholesterol concentrations, in the range for which pharmaceutical intervention is not absolutely required.

In several animal studies in which AGE was administered in conjunction with cholesterol loading or a diet conducive to cholesterol elevation, it produced far greater reductions (>30%) in serum cholesterol (24). This difference in efficacy could be due to a difference in cholesterol homeostasis, slightly higher doses used in animal studies, and/or the diet. If the diet is the cause of the difference, then AGE could produce a larger cholesterol-lowering effect among individuals consuming excessive amounts of cholesterol or a diet that is conducive to hypercholesterolemia. The subjects in this study were advised to follow the National Cholesterol Education Program Step I diet, which is hypocholesterolemic.

It was reported that an odor-modified liquid garlic extract produced transient elevations in total cholesterol and triacylglycerol concentrations and the ratio of LDL to very-low-density lipoprotein during initial supplementation (7). This was later followed by a significant reduction below baseline values. We did not observe this phenomenon in our study but the average cholesterol concentration in our study population was considerably less than that reported in the above study. Neither did we observe the HDL cholesterol-elevating effect of the liquid garlic extract reported by the same authors.

Our results show that AGE is a safe supplement over extended periods of time and that it does not alter blood counts or change blood chemistry or thyroid function. Furthermore, our double-blind crossover study showed that garlic supplementation can produce significant reductions in total as well as LDL cholesterol but did not change HDL cholesterol and triacylglycerol concentrations. In addition, blood pressure is beneficially affected by garlic administration.

REFERENCES