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Effect of garlic supplementation on plasma lipids in hypercholesterolemic adults

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**EFFECT OF GARLIC SUPPLEMENTATION ON PLASMA LIPIDS IN
HYPERCHOLESTEROLEMIC ADULTS**

A Thesis

Presented to

The Faculty of the Department of Nutrition and Food Science

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Lorraine Chatterjee

May, 2000

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Abstract

Effect of Garlic Supplementation on Plasma Lipids in Hypercholesterolemic Adults

By

Lorraine Chatterjee

Health claims regarding the cholesterol lowering effect of garlic are widespread. However, clinical trial data are inconsistent. A double-blinded, randomized, placebo-controlled, parallel design trial was conducted in an outpatient setting to evaluate the effect of two doses of a commercially available garlic supplement on plasma low-density lipoprotein cholesterol (LDL-C), in moderately hypercholesterolemic adults over a 12-week period. Secondary outcome measures included other plasma lipids and blood pressure.

Fifty-one adults, 51.8 ± 8.3 years of age, were randomized to a garlic-licorice botanical blend providing either 1000mg/day (full-dose) or 500mg/day (half-dose) garlic powder or a placebo. Plasma lipids and blood pressure were assessed semimonthly. During the study adherence, dietary intake, body weight and physical activity were comparable in the three treatment arms. The LDL-C decrease of 6.1% in the full-dose group was statistically insignificant ($p>0.05$). No significant differences were observed for other plasma lipids and blood pressure.

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Preface

Chapter one that includes the introduction and review of literature and chapter three that includes the summary and recommendations for future research have been written in the American Psychological Association format. Chapter two is written in publication format for submission to the American Journal of Clinical Nutrition.

CHAPTER 1: INTRODUCTION AND REVIEW OF LITERATURE

Introduction

The use of garlic in medicinal preparations has been reported throughout history. Claims of prophylactic properties of this widely quoted herb have been based on garlic's extensive use in traditional Chinese and Ayurvedic medicine (Fenwick & Hanley, 1985b). Early interest in garlic focused on its antiviral and anti-bacterial properties. Current research suggests garlic may have a possible role in reducing cardiovascular risk (Santos & Johns, 1995; Jain, Vargas, Gotzkowsky & McMahon, 1993; Steiner, Khan, Holbert & Lin, 1996) by normalizing lipid concentrations. Two meta-analyses of clinical trials support garlic as a lipid-lowering agent (Silagy & Neil, 1994; Warshafsky, Kamer & Sivak, 1993).

Complimentary and alternative medicine (CAM) has a definitive presence in the U.S. health care system. It encompasses non-traditional medical interventions that are generally not considered part of the US health care system and includes the use of herbs and plants with medicinal potential, such as garlic. Rapid growth in the popularity of over-the-counter herbal compounds has increased sales to \$1.5 billion in 1997 from \$53 million in 1992 (Stavish, 1998). A follow-up national survey of the trends in CAM use in the United States reported that the prevalence increased by 25% between 1990 and 1997 (Eisenberg et al., 1998). The increase was attributable to the increase in the proportion of the population using alternative therapies rather than increased visits per patient. In 1997, a total of \$27 billion was estimated as the out-of-pocket expenditure for alternative therapies. A review paper on the referral and personal practice rates for CAM among

main stream physicians is indicative of a growing belief of the usefulness of these modalities. Some of the reasons cited by physicians for the use of or referral for CAM therapies included lack of response to conventional treatment, belief in efficacy and fewer side effects (Astin, Marie. Pelletier, Hansen & Haskell, 1998).

Management of plasma cholesterol levels continues to be a cardinal issue in cardiovascular health. Behavioral and pharmacological approaches have been established for lipid management (Wood, Stefanick, Williams, Haskell, 1991, Shepherd, et al., 1995, Haskell, et al., 1994). Lipid lowering drugs have been associated with undesirable side effects and non-cardiovascular morbidity. This has led to an increase in non-pharmacological therapies for lipid management. Garlic is being rigorously researched as a promising approach for improving lipid concentrations, plasma viscosity and platelet aggregation with few adverse effects (Ernst, Weihmayr & Matrai, 1985).

Significance of the Study

Evidence that garlic reduces cardiovascular risk factors is accumulating but is incomplete. A review article (Kleijnen, Knipschild & TerRiet, 1989) concluded that while large doses of fresh garlic produced beneficial effects on cardiovascular risk factors, data for commercial supplements were less convincing. Although the results of the meta-analyses (Silagy & Neil, 1994; Warshafsky et al., 1993) appear compelling, many of the individual studies were flawed with methodological shortcomings, inadequate reporting of data collection or analyses and lack of control for potential confounders as well as publication bias. Also, a possible dose-response to garlic preparations and the pattern of change in lipid profile during therapy has not been

addressed. Limited data suggest that garlic supplementation may transiently elevate cholesterol levels during the initial weeks of therapy due to mobilization of cholesterol from tissues (Bordia, 1981; Lau, Lam & Wang-Cheng, 1987).

Objective

The purpose of this study was to examine the effect of a commercial garlic–licorice botanical blend on the lipid profile of mildly hypercholesterolemic adults. The study involved three treatment arms. One group was given garlic tablets containing 1000 mg garlic powder/day, another received the supplement at half dose (500 mg garlic powder/day) and the placebo group received a blank comprising mostly of cellulose and dextrose. The primary hypothesis of the study was that 12 weeks of garlic supplementation would lower plasma total and low-density lipoprotein cholesterol (LDL-cholesterol) levels in modestly hypercholesterolemic adults. It was also hypothesized that plasma cholesterol would exhibit a dose-response in the three treatment arms. The group administered the full garlic dose would have a proportionately higher response than the intermediate dose, relative to the placebo group. The lipid parameters were analyzed bimonthly, to track a pattern of change during the intervention.

Review of Literature

Active Compounds in Garlic

The Latin name for garlic, *Allium*, is derived from the Celtic word “al”, meaning pungent. The species name *Sativum* translates as cultivated or sown. In 1944, chemist Cavallito isolated an unstable, odorous, sulfur containing compound in garlic and named it allicin. There is widely accepted evidence that the main known pharmacologically

active ingredient in garlic is allicin (Lawson & Block, 1997). Allicin is not present in raw garlic but is produced rapidly from the odorless precursor alliin, S-allylcysteine sulfoxide, by the action of the enzyme allinase. This enzyme is released from garlic by mechanical disruption of the cloves such as crushing and cleaves alliin to allicin. It is estimated that 3.7 mg of allicin per gram of garlic is released by enzymatic action. Although allicin is the most potent organosulfur constituent of garlic, it is not found in blood. Studies suggest that it does not reach target organs after garlic consumption (Freeman & Koder, 1995). Allicin and some of its important transformation products are highly unstable at room temperature and are metabolized primarily to a series of derivatives or thiosulfinates. The half-life of allicin in water at room temperature is four days (Calvey, et al., 1997). Commercially, the medicinal value of garlic preparations is evaluated on the basis of its allicin yield or allicin-producing potential, that is, alliin and allinase activity. A High Pressure Liquid Chromatography (HPLC) analysis demonstrated that only traces of allicin were detected, following incubation in blood, for five minutes. The results of the analysis suggested that allicin is not the principle metabolite of garlic in vivo. Furthermore, the analyses revealed that the allicin content and allicin-producing potential of several commercial garlic preparations was found to be less than the detectable limit of one ppm under simulated gastric conditions. Variable allicin potentials were observed in different brands of products as well as among different batches of the same brand (Freeman & Koder, 1995). The exact composition of commercial garlic preparations is mostly unknown. Heat processing may cause a decrease or loss of active compounds in garlic. The differences in the therapeutic effect

may be due to a thirteen-fold variation in the content of organosulfur compounds in garlic. The proportion of different thiosulfinates in species of garlic varies with climatic conditions, differences in cultivation, harvesting and post-harvest processing. Varieties of garlic harvested in cooler regions showed a higher allyl to methyl ratio than garlic grown in warmer climates (Block, Naganathan, Putnam & Zhao, 1992). Varying enzymatic and chemical reactions that occur during the commercial preparation of garlic products may result in different end products (Broche, Platt & Dorner, 1990). It is known that several nutrients have to be metabolized in the body to produce pharmacological activity. Lawson and Wang (1993) stated that it is the metabolites of allicin that are the ultimate active agents. Allinase is completely and irreversibly inhibited by gastric acid in the stomach (Lawson & Hughes, 1991). Hence, standardized garlic preparations should be available as enteric-coated formulations.

Ajoene, a garlic-derived polar compound, was reported to be a potent antithrombotic agent. This compound was found to inhibit aggregation induced by various platelet stimulators, ADP, epinephrine, collagen, arachidonic acid, thrombin, platelet-activating factor and calcium ionophore (Apitz-Castro, Cabrera, Cruz, Ledezma & Jain, 1983). Ajoene is reported to alter platelet membranes by capturing sulfhydryl groups. The ability of garlic oil to inhibit aggregation of blood platelets has been ascribed to the presence of allyl methyl trisulfides or diallyl trisulfides (Block, Ahmad, Catalfamo, Jain & Apitz-Castro, 1986).

Garlic Sales and Consumption

Since 1969, global cultivation and yield of garlic has steadily increased. In the U.S., the crop is valued at over \$ 30 million. Gilroy, California has the largest garlic dehydration plant in the world (Fenwick & Hanley, 1985a). According to the USDA, in 1994, California alone produced approximately 500 pounds of garlic. Garlic consumption is being promoted as a charm against heart disease causing its sale figures to more than double in the past decade. The Nutrition Business Journal (1998) quoted garlic as one of the top herbal remedies in 1997, which accounted for sales of \$200 million, up 33% from 1995 sales. Commercially garlic preparations are available as freeze-dried, air-dried, essential oil, aged extracts and botanical blends (Myers & Smith, 1997).

A national survey in the U.S. reported that unconventional therapies, including herbs such as garlic, are used as adjuncts to conventional medical treatment (Eisenberg et al., 1993). A survey in Australia revealed that women used significantly more herbal supplements than men. Users were also reported to be educated and belonging to the upper socio-economic strata (MacLennan, Wilson & Taylor, 1996).

Mechanistic Evidence for Beneficial Effects of Garlic

Several mechanistic studies have consistently reported an inhibitory effect of garlic on cholesterol biosynthesis. Chi, Koh and Stewart (1982) reported that lyophilized garlic decreased plasma cholesterol in male rats fed cholesterol and lard diets. Plasma triglycerides decreased only in the lard-fed group. The authors reported that garlic supplementation enhanced the excretion of neutral steroids and bile acids and possibly

decreased activity of lipogenic enzymes glucose-6phosphate dehydrogenase and malic dehydrogenase.

Garlic powder was added to smooth muscle cells cultured from atherosclerotic plaques of human aorta. During a 24 hour incubation period, the garlic powder extract significantly lowered intracellular levels of cholesterol and cholesteryl esters.

Proliferative activity of the cultured cells, as measured by the incorporation of labelled [^3H] thymidine into cellular DNA, was inhibited. The anti-atherosclerotic effect was observed at a therapeutic dose of $10\mu\text{g/ml}$ of garlic. To demonstrate the anti-atherogenic effects of garlic, serum from coronary artery disease patients given a single dose of 300mg garlic, was added to cultured cells from normal human aortic intima. Cholesterol accumulation and cell proliferation were substantially reduced (Orekhov, Tertov, Sobenin & Pivovarova, 1995).

Studies using hepatocytes (Gebhardt, 1993; Yeh & Yeh, 1994) have demonstrated that garlic supplemented diets resulted in a dose-dependent inhibition of cholesterol biosynthesis at different enzymatic steps. Gebhardt using aqueous extracts of garlic observed that at low concentrations of garlic, sterol biosynthesis from acetate decreased 23% in rat hepatocytes and 28% in human hepatoma cells by depressing the cholesterologenic enzyme, 3-hydroxy-3-methyl-glutaryl-CoA (HMG CoA) reductase. There was no evidence of the inhibitory effect when labeled mevalonate was used as the precursor instead of acetate, indicating that the inhibition occurs at an early enzymatic step prior to the formation of mevalonate. At high concentrations of garlic extract (too high to be reached during normal or even long-term garlic consumption), inhibition also

occurred at later stages of cholesterol biosynthesis resulting in the accumulation of sterol precursors, lanosterol and 7-dehydrocholesterol. While alliin the main sulfur-containing compound of garlic was ineffective, if converted to allicin, it exerted changes in sterol accumulation. Yeh and Yeh corroborated the above findings, by demonstrating that depressed rates of hepatic cholesterogenesis from the substrate acetate were related to a lowered activity of the rate-limiting enzyme reductase and possible inhibition of acetyl-CoA synthetase. The triglyceride lowering effect of garlic appeared to be due to the inhibition of de novo fatty acid synthesis. The garlic extracts were also effective in reducing the incorporation of glycerol into diacylglycerol and phospholipids but did not inhibit the rate of glycerol incorporation into triglycerides when oleic acid was present in the culture medium.

In a later study Gephardt and Beck (1996) used allicin as well as various garlic-derived organosulfur compounds to compare the inhibitory effect on hepatocellular cholesterol biosynthesis in rats. Although allicin was the most potent inhibitor, the influence of the other derived products including diallyl sulfide and allyl mercaptan and vinyl-dithins varied considerably. Using a modified liver homogenate model (Sendl, Schliack, Loser, Stanislaus & Wagner, 1992) it was demonstrated that multiple thiosulfinates, ajoene and dithines from garlic extracts reduced serum cholesterol synthesis by 37 to 72 percent.

The anti-aggregatory activity of garlic oil correlated with decreased thromboxane and prostaglandin biosynthesis through the inhibition of platelet fatty acid oxygenases. The influence of garlic on arachidonic acid metabolism was dose-dependent and involved

inhibition of the cyclooxygenase enzyme, that catalyzed the formation of prostaglandins, as well as the lipoxygenase pathway through a product redistribution (Vanderhoek, Makheja & Bailey, 1980). Garlic treated Wistar rats showed decreased incidence of severe arrhythmias like ventricular tachycardia and fibrillation. The size of the myocardial ischemia was smaller ($31.7 \pm 2.4\%$) in the garlic treated than in the untreated control group ($40.9 \pm 2.3\%$). Garlic therapy did not induce significant changes in the fatty acid composition of myocardial membranes. In the absence of thrombocytes, the prostaglandin-thromboxane antagonism did not play an essential role in the cardioprotection observed (Isensee, Rietz & Jacob, 1993).

Animal Studies

Data from animal studies have supported the above-cited mechanistic evidence, for a possible hypolipidemic effect of garlic. A prospective placebo-controlled animal study was conducted to study the effects of allicin on serum lipid levels in rabbits (Eilat et al., 1995). Total cholesterol (TC) concentrations decreased 19% and LDL-C decreased 20% in the treatment group. However, 10 weeks following the addition of allicin to the high cholesterol diets that were fed to the rabbits, the difference in lipid levels between the control and treatment group was no longer evident. It was suggested that the prolonged feeding of dietary cholesterol at high concentrations induced non-physiological hypercholesterolemia in the animals. Alternatively, it was postulated that allicin may have had a short-acting effect on serum lipid concentrations. Kamanna and Chandrasekhara (1982) reported that garlic powder added to atherogenic diets at 2.0 % levels, produced a significant decrease in liver and serum cholesterol in albino rats, which

was not observed at lower levels of supplementation. Bordia and coworkers (1977) reported that garlic oil fed to rabbits (one gram raw garlic/kg body weight) lowered serum cholesterol and triglyceride levels. It also reduced aortic atheroma by approximately one half in a model of experimentally induced atherosclerosis. In another controlled study, chickens fed garlic powder demonstrated altered lipid and cholesterol metabolism. The supplementation caused a reduction in plasma, liver and muscle cholesterol. HMG CoA reductase and alpha hydroxylase activity were significantly reduced (Konjufca, Pesti & Bakalli, 1997).

More recently it was demonstrated that in cholesterol fed rabbits 'Kyolic' containing aged garlic extract inhibited thickened lipid-filled lesions, in the intima of carotid arteries, de-endothelialized by balloon catheterisation. This effect was not evident in the rabbits fed a standard diet (Efendy, Simmons, Campbell & Campbell, 1997).

Lipid-Lowering Effect in Humans

The antilipemic effect of garlic preparations has been researched in randomized, controlled clinical trials. Bordia (1981) studied the effect of garlic supplementation on healthy individuals and patients with a history of coronary heart disease (CHD). Serum cholesterol (17%) and triglycerides (20%) were significantly lowered in the group of 20 healthy volunteers after six months of garlic supplementation. The dose was equivalent to 30 g/day fresh garlic (0.25 mg garlic oil/kg body weight) in the form of gelatin capsules. The high-density lipoprotein cholesterol (HDL-C) showed an increase. The study also reported that in the case of the 62 patients diagnosed with CHD, an initial transient rise in serum cholesterol was observed followed by a significant decrease

($p < 0.05$) in levels after three months of supplementation with the garlic capsules. Lau et al. (1987) reported a similar response in the serum lipid values of hypercholesterolemic adults, using an odor-modified garlic preparation (250mg dry weight). The transient increase in serum triglycerides, total and LDL cholesterol was evident after one month of therapy. Garlic did not decrease the serum lipids in participants whose initial serum total cholesterol were within normal limits. This finding was corroborated in later studies (Broche, et al., 1990; Mader, 1990) which reported that the decrease in total cholesterol in response to standardized garlic powder tablets (600 and 800mg/day respectively) was greater in participants with higher initial values. Also, the reduction in triglycerides was evident only in the case of elevated total triglycerides. In a study conducted by Gadkari and Joshi (1991), a significant decrease ($p < 0.001$) in serum cholesterol and fibrinolytic activity was reported following ingestion of 10g raw garlic each day for two months. The 50 subjects selected aged 17 to 22 years, had never consumed garlic before and were reported to have had normal lipid profiles.

Standardized dried garlic preparations with allicin-producing potential have been used in several clinical trials. Harenberg, Giese and Zimmermann (1988) provided 20 hyperlipoproteinemic outpatients dried garlic (600mg/day) as sugar coated standardized pills for a month. The garlic was dried at 50° C. Serum total cholesterol and fibrinogen decreased by 10 percent. The decrease in systolic and diastolic blood pressure was significant ($p < 0.01$). Serum HDL-cholesterol, LDL-cholesterol and triglycerides did not change during the intervention. The multi-centric study reported by Mader (1990) showed a 12% drop in serum total cholesterol and 17% in serum triglycerides. The

subjects were moderately hypercholesterolemic adults who received 800 mg/day standardized garlic powder tablets or a placebo for four months. In another clinical study a modest reduction in total cholesterol (6%) and LDL-cholesterol (11%) in the treated group and 3% in the placebo group was seen with 900 mg/day standardized garlic powder tablets. The subgroup with the highest baseline total cholesterol ranging from 250-300mg/dl showed the greatest response, with a reduction of 14% in serum cholesterol at the end of the study. The hypolipidemic effect was evident as early as four weeks following therapy and it progressively increased. There was a non-significant change in HDL-cholesterol, serum triglycerides and blood pressure after the 12-week intervention period (Jain, et al., 1993). Other studies using similar doses of garlic powder tablets (900mg/day) were associated with significantly lower serum total and LDL-cholesterol, triglycerides and blood pressure than the placebo group (Voberg & Schneider, 1990; Zimmermann & Zimmermann, 1990). In a 16-week study, Voberg and Schneider reported that a self-evaluation questionnaire administered to the population of 40 hypercholesterolemic outpatients revealed a greater feeling of wellbeing in the garlic treated group. The Zimmermann study was conducted for three weeks among 23 hyperlipoproteinemic hospitalized patients.

An open randomized study compared the effects of garlic powder and garlic oil on blood lipids and blood pressure. After 16 weeks of treatment it was observed that standardized garlic powder (equivalent to 0.6% allicin) was more effective in reducing total cholesterol than garlic oil (11% and 3% decrease respectively). The reductions in LDL-cholesterol in the two groups was similar (16 and 15 percent respectively).

Significant changes in blood pressure were reported using garlic powder (De Santos & Johns, 1995). In an open study 20 hypercholesterolemia patients were prescribed hypocaloric diets. Ten patients received in addition 21g garlic powder equivalent to 64g fresh garlic a day for four weeks. Serum triglyceride and total cholesterol levels were significantly lower in the group that received garlic (Ernst, et al., 1985).

In a double-blind crossover study, Steiner and coworkers (1996) compared the effect of aged garlic extract (AGE) with a placebo, on blood lipids in 41 moderately hypercholesterolemic men. Participants were instructed to adhere to the National Cholesterol Education Program (NCEP) Step 1 diet for the duration of the study. After a four- week baseline period, they were randomly assigned to either garlic capsules (nine/day) containing 7.2 g/day AGE or a placebo. The first intervention period lasted for 24 weeks and thereafter the participants were put on the alternate treatment for 16 weeks. Baseline blood lipids and blood pressure were measured weekly during the baseline period and subsequently at each monthly visit. Consumption of AGE significantly reduced total cholesterol and LDL-cholesterol ($P=0.0001$), with a maximum effect evident after three months of supplementation.

In a randomized, placebo-controlled, double-blind study garlic was administered as a combination product with ginkgo (200mg garlic extract/day containing 5.72mg alliin). The study was conducted for two months to investigate whether standardized garlic tablets prevented deterioration in lipid levels during the holiday season in December compared to a placebo. There were no significant changes in serum total cholesterol in both groups at the end of the study. However, 35% in the garlic-ginkgo

group showed an improvement in total cholesterol compared to 20% in the placebo group. The mean reduction in the garlic-ginkgo group was 10.4 percent (Kenzelmann & Kade, 1993).

A study examined the effects of garlic and fish-oil supplementation (alone and in combination) on serum lipids and lipoproteins in 50 hypercholesterolemic male subjects. Garlic supplementation with and without fish-oil significantly decreased total (12.2 and 11.5 percent respectively) and LDL cholesterol (9.5 and 14.2 percent respectively). The garlic groups also reported significantly lower ratios of total to HDL cholesterol and LDL to HDL cholesterol. The authors concluded that a combination of garlic and fish-oil was effective in reducing the moderate rise in LDL cholesterol induced by fish-oils (Adler & Holub, 1997).

Three meta-analyses have compiled the results of primary clinical trials on the lipid lowering effect of garlic (Kleijnen et al., 1989; Warshafsky et al., 1993; Silagy & Neil, 1994). Kleijnen et al. compared the effects of commercial garlic preparations to fresh garlic. Five out of six trials that used fresh garlic (0.25–1.0 g/kg body weight) or freshly made garlic extracts either as a single dose or over one to two months, demonstrated a lowering of serum cholesterol. There was a distinct inhibition of platelet aggregation and an increase in fibrinolytic activity. When commercially available garlic preparations were tested (in five randomized studies) contradictory results were reported.

Warshafsky et al. (1993) and Silagy and Neil (1994) indicated that the administration of 600-900 mg/day garlic preparations, over 4 to 12 weeks, resulted in an average reduction of total cholesterol of 9 to 12 percent. Triglyceride levels fell 8 to 27

percent. The Warshafsky analysis of five published, randomized, placebo-controlled studies was conducted to assess, the size and consistency of the hypolipidemic effect of garlic in patients with elevated levels of serum cholesterol (> 200 mg/dl). This meta-analysis of homogenous trials suggested that a half to a clove of garlic decreased total cholesterol by 9% in the groups of patients studied. To determine the effect of garlic on serum lipids and lipoproteins relative to placebo, the Silagy and Neil meta-analysis reviewed randomized, controlled trials of garlic preparations of at least four weeks duration. Sixteen trials, with data from 952 subjects were included. The effect of garlic on blood lipids was measured as the difference between the mean change in values in the treatment and placebo groups. Total cholesterol decreased by 12% with garlic therapy beyond the final levels achieved by placebo alone. There was no significant difference in the size of the reduction, across the dose range of 600-900 mg of standardized dried garlic powder preparations taken daily.

The clinical trial data are not entirely consistent. While many studies have reported a substantial hypocholesterolemic effect of garlic others have reported no detectable lipid response. Arora and Arora (1981) studied 17 healthy males and 11 randomly selected patients suffering from ischemic heart disease (IHD). Garlic (extracts of 50 g of raw garlic) and clofibrate was fed individually along with a high-fat diet to each subject. Each intervention lasted for a week. The only beneficial effect of garlic was an observed decreased coagulation time and an increase in fibrinolytic activity following a dietary fat load. In another study Arora, Arora and Gupta, (1981) concluded that 12 weeks of garlic therapy (six garlic oil capsules, each containing 0.625 mg garlic

oil) did not produce changes in serum total cholesterol, triglycerides, plasma fibrinogen levels and coagulation time in IHD or healthy subjects. A dried garlic preparation was administered in two doses approximately 600 mg and 1350 mg per day to hyperlipoproteinemic patients (Luley, Lehmann-Leo, Möller, Martin & Schwartzkopff, 1986). The six-week study was placebo-controlled, randomized, double-blinded and lipid parameters were measured every three weeks. Neither dosage of dried garlic showed significant effect on the parameters measured.

Simons and coworkers (1995) have found little lipid-lowering activity as a result of garlic ingestion. Twenty-eight volunteers with a history of hypercholesterolemia were subject to strict dietary supervision (NCEP diet) and assessment. After a baseline period of four weeks, subjects randomly received either 900 mg/day of Kwai garlic tablets or a matching placebo for 12 weeks. They crossed over to the alternative treatment for the remainder 12 weeks after a four-week washout period. The analysis was performed on an intention-to-treat basis. There were no significant changes in plasma cholesterol, LDL and HDL cholesterol, plasma triglycerides and blood pressure between the periods on garlic tablets and placebo. A trial conducted in the United Kingdom (Neil et al., 1996) also demonstrated that garlic was less effective in reducing total cholesterol than suggested by previous meta-analyses. One hundred and six patients completed the 24 week long study. They were randomly assigned either to 900 mg/day of garlic tablets or a placebo. Eight weeks after randomization, compliance and were checked. Lipid parameters were measured at baseline and at the end of the study. At the end of the six-

month trial, there were no significant group differences in the mean lipid concentrations or in the mean individual changes in lipid concentrations.

A recent crossover trial (Berthold, Sudhop & Bergman, 1998) used steam-distilled garlic oil to test the effect on serum lipids in 25 adult participants with elevated LDL cholesterol. Each phase of the crossover lasted 12 weeks. The dosage used was 10 mg/day which is equivalent to 4 to 5 g of fresh garlic or 4000 allicin-equivalents per day. Compliance was rated high, averaging 98 percent. Dietary intakes during each of the two treatment phases were assessed using 7-day food records and were not found to differ. Minimal side effects were reported and none serious enough to influence the study outcome. The investigation did not detect any significant lipid changes in the two treatment phases and concluded that steamed-distilled garlic oil was ineffective in lowering elevated cholesterol in hypercholesterolemic adults. In addition an examination of biochemical markers for cholesterol metabolism demonstrated no significant differences between groups in cholesterol synthesis or absorption.

Isaachsohn and coworkers (1998) conducted a randomized, placebo-controlled, parallel design, 12-week intervention to determine the lipid-lowering effect of garlic tablets. Fifty hypercholesterolemic men and women, with a mean $\pm$ SD LDL-cholesterol of 172 ± 14 mg/dl were assigned either to 900 mg dried garlic powder or a placebo each day. Monthly analyses of diet records indicated no group differences in nutrient intake and weight remained stable and comparative in the two groups. Compliance as estimated by a pill count was approximately 90%. Minimal side effects were reported and a total of

eight participants dropped out four in each group. No significant differences were reported for LDL-C or any of the other plasma lipids measured.

A critical evaluation of the studies reporting a beneficial effect of garlic supplementation revealed that in the case of the two meta-analyses (Warshafsky et al., 1993; Neil et al., 1996), several of the primary trials included in the data, had methodological shortcomings. These included poorly characterized subject groups, lack of description of study methodology, possible underreporting of side effects as well as insufficient statistical power for calculating an effect size and failure to undertake an 'intention-to-treat' analysis. A funnel-plot analysis showed there were fewer published negative studies in these meta-analyses than expected which may have resulted in a false positive outcome (Beaglehole, 1996). The criteria for including and excluding primary trials were established after the data were extracted and reviewed (Lerner & Hulley, 1994). The crossover design of the Steiner et al. study (1996) with no washout period between each treatment was complex to analyze and interpret due to the elusive problem of carryover effects. The authors provided no justification for the difference in duration (24 and 16 weeks) in each crossover phase although they did report that the maximal effect was evident in 12 weeks. There appears to be a substantial difference in the baseline levels of LDL-C, when the study population was randomized to either the garlic or placebo group following a run-in of four weeks using the NCEP Step One guidelines. The large number of tablets (nine/day) may have been unrealistic resulting in poor adherence. The blinding was ineffective with 70% of those on the garlic supplement correctly identifying the assigned treatment. The substantial dropout rate of 21% limits

the generalizability of the findings. Jain et al. (1993) included smokers and at least three subjects on anti-hypertensive drugs. No data was presented to document control of confounders such as diet and physical activity as well as compliance of the subjects. Funding for the study was provided by the supplier of the garlic tablets giving rise to a potential for a conflict of interest. The study reported by Zimmermann and Zimmermann (1990) lacked a control group and randomized treatment. The overall quality of several studies (Bordia, 1981; Gadkari & Joshi, 1991; Harenberg et al., 1988; Warshafsky et al., 1993) was questionable as supplement compliance, effectiveness of blinding participants and investigators, side effects and tolerance were not measured. Bordia et al. (1996) provided little documentation of the conduct of the study and therefore lacked rigor and scientific merit. In the case of the well-controlled study conducted by Adler and Holub (1997) no apparent conflict of interest existed as funding for the study was not provided by the manufacturer of the garlic tablets. However the small sample size in the garlic (n=13) and placebo (n=12) groups requires that the conclusions be approached cautiously.

Reviewing the trials that reported no demonstrable effects of garlic supplementation on blood lipids, lipoproteins and blood pressure two of the studies (Simons et al., 1995; Neil et al., 1996) conceded that they may have failed by chance to detect a true effect in this trial (type 2 error). The results of the Neil study were included in a reanalysis of their previous overview (Silagy & Neil, 1994). The pooled estimate for the effect of standardized garlic compared to placebo remained statistically significant, but was reduced in magnitude. The study, although the largest of any of the trials

reported here, with 106 hypercholesterolemic patients, lacked adequate follow-up to monitor compliance and tolerance. Compliance was poor with roughly half the participants taking less than 75% of their supplements. Potential lifestyle confounders such as diet and exercise were not addressed. In the meta-analyses by Kleijnen et al. (1989) although four of the five randomized studies that reported to use garlic supplements were blinded only one trial monitored for tolerance and compliance.

Garlic and Blood Pressure

Cardioprotection entails more than lipid reduction (Myers & Smith, 1997). Hypotensive effects of garlic were attributed to its vasodilatory properties (Keisewetter, et al., 1991; Wolf, Reim & Jung, 1990). Data from studies reviewed earlier (Jain et al., 1993; Simons et al., 1995; Zimmermann & Zimmermann, 1990) suggested that garlic consumption did not significantly alter blood pressure (BP) in a normotensive population. Standardized garlic preparations were reported to be beneficial to patients with moderate hypertension (Adler & Holub, 1997; Santos & Johns, 1995; Harenberg et al., 1988; Silagy & Neil, 1994; Steiner et al., 1996; Voberg & Schneider, 1990) when administered for 12 to 16 weeks. Auer et al. (1990) reviewed a randomized, placebo-controlled, double-blind trial conducted by 11 general practitioners on 47 mildly hypertensive patients. After a 14-day acclimatization period, the patients were randomly assigned to 600 mg/day powdered garlic supplements or a placebo for 12 weeks. The supine systolic BP fell significantly ($p < 0.05$) and there was a 13% reduction in the corresponding diastolic BP in the garlic group compared with a 4% drop in the placebo group.

In an open-label study McMahon and Vargas (1993) reported on nine severely hypertensive patients (diastolic BP>115mm Hg) who were administered a megadose of garlic at 2400 mg. Sitting and erect diastolic blood pressures appeared to be lowered significantly 5 to 14 hours after the dose was administered. Despite the lack of statistical significance, a definite trend was observed in the systolic BP, which may be of greater significance in a larger and more controlled sample population.

The Auer multi-center trial (1990) although well designed, included diabetics and 21 on-study patients were taking a total of 27 other drugs in addition to the garlic supplement. This may have interfered with the true outcome of the study. The open-label study (McMahon & Vargas, 1993) lacked a control group.

Garlic and Antiplatelet Activity

There is considerable evidence, that platelets play a significant role in development of atherosclerosis. Increased platelet activity is present in smokers, patients with hyperlipidemia and hypertension. Platelet aggregates deposit in the vascular endothelium causing narrowing of the lumen. Enzymes, cyclooxygenase and lipoxygenase catalyze the formation of metabolites such as prostaglandin endoperoxide and thromboxane from arachidonic acid. These metabolites are known to induce platelet release and aggregation (Lau, 1989). Components of garlic inhibit the cyclooxygenase and lipoxygenase pathways, thereby blocking thromboxane synthesis (Agarwal, 1996). Researchers reported a significant increase in arachidonic acid in erythrocyte phospholipids following garlic administration (Barrie, Wright & Pizzorno, 1987). This was thought to be due to blockage of the arachidonic acid cascade to thromboxane A₂.

One of the components identified by mass spectrometry and nuclear magnetic resonance was methyl allyl trisulfide (Agriga, Oshiba & Tamada, 1981). Ajoene derived from garlic extracts inhibits platelet aggregation from all known inducers (Block, et al., 1984).

The dose-related acute effect in healthy individuals and the long-term effect of garlic oil in both healthy and CAD subjects were examined. It was observed that large doses of garlic oil (10 to 14 capsules) administered in one dose was not effective in inhibiting platelet aggregation. However a lower dose (six capsules) became effective in long-term administration of a month (Bordia, Verma & Srivastava, 1996).

In a placebo-controlled, double-blinded study Kiesewetter and coworkers (1991) observed significant positive effects through the use of 800 mg of garlic powder per day for four weeks. Spontaneous thrombocyte aggregation was measured in 30 participants, mean age 23 years. It decreased significantly by 56% in the active substance group. Also after four weeks of treatment, erythrocyte velocity in the nail fold capillaries was significantly elevated, indicating that microcirculation of skin improved. Plasma viscosity decreased by 3.2% while no change was observed in the placebo group.

A decrease in spontaneous thrombocyte aggregation was demonstrated among patients with peripheral arterial occlusive disease. Thirty-two patients who received 800 mg/day were evaluated in a randomized, placebo-controlled, double-blinded study for 12 weeks. The efficacy was not detectable until 4-6 weeks of garlic therapy (Kiesewetter et al., 1993).

Eight healthy male volunteers between the ages of 40 and 50 consumed a clove of fresh garlic after an overnight fast for 16 weeks (Ali & Thomson, 1995). Thromboxane

levels decreased approximately 20% after four weeks of garlic consumption and a further decrease (80%) was observed after 16 weeks.

Kiesewetter et al. (1991) proposed that garlic would be useful in cases of acetylsalicylic acid intolerance, due to its potential vascular protection role. However, the study did present a few limitations. It was conducted for a relatively short duration of four weeks and tolerance and blinding techniques were not described. There was good follow-up with explorative parameters measured on a weekly basis. Ali and Thompson (1995) suggested that small amounts of fresh garlic consumed over a long period may result in decreased thrombosis thus influencing the development of atherosclerosis. However the sample size was limited and a description of the methodology used to recruit volunteers, as well as of the statistical analysis was lacking.

CHAPTER 2

JOURNAL ARTICLE

Title Page

Effect of garlic supplementation on plasma lipids in hypercholesterolemic adults

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Short title/running head:

Garlic and Plasma Cholesterol

ABSTRACT

Background: The role of cholesterol reduction in the prevention and management of CVD is well established. Health claims regarding the cholesterol lowering benefits of garlic are widespread. However, the clinical trial data are inconsistent.

Objective: The study was conducted to evaluate the effect of two doses of a commercial garlic supplement on plasma LDL-C, compared to a placebo, in moderately hypercholesterolemic adults (baseline LDL-C= 157.4 ± 18.7 , mean $\pm$ SD) over 12 weeks. Secondary outcome measures included other plasma lipids and blood pressure.

Design: Fifty-one adults, aged 51.8 ± 8.3 years participated in a double blind, randomized, placebo-controlled, parallel treatment trial conducted in an outpatient research clinic. They were randomized to a placebo or a garlic-licorice botanical blend providing 500 or 1000 mg garlic powder/day. Plasma lipids and blood pressure were assessed every two weeks.

Results: During the study adherence, dietary intake, physical activity and body weight were comparable in the three treatment arms. The absolute mean changes in LDL-C were 0.0 ± 4.3 , $+1.4 \pm 4.8$, and -10.1 ± 6.8 mg/dL for the placebo, half-dose and full-dose, respectively. The LDL-C decrease of 6.1% in the full-dose group was not significantly different from that in the other groups ($p=0.5$). No significant differences were observed for secondary measures.

Conclusion: Garlic supplementation in a moderately hypercholesterolemic, normotensive sample of adults had a marginal to negligible effect on plasma lipids and blood pressure that was not statistically significant between groups. Although not efficacious in this

study, garlic supplementation may be warranted for other health outcomes not assessed here.

Key Words: Cholesterol, garlic supplements, hyperlipidemia, allicin, low-density-lipoprotein cholesterol, clinical trial

INTRODUCTION

Management of plasma cholesterol concentrations continues to be a cardinal issue in cardiovascular disease prevention. Hygienic and pharmacological approaches have been established for lipid management (1). A growing interest in complementary and alternative medicine (2,3) has led to an increasing number of non-pharmacological therapies for lipid management. Among these is garlic, which in 1997 was one of the top herbal remedies in consumer demand. That year garlic supplements accounted for sales of \$200 million dollars, up 33% from 1995 sales (4). Garlic has been reported to have numerous prophylactic and remedial benefits, including decreasing plasma cholesterol concentrations and blood pressure, enhancing immune function, decreasing platelet aggregation and blood coagulation, and improving vasodilation (5).

A number of clinical trials have examined the role of garlic in lipid management. Although several reviews and at least two meta-analyses have been conducted (6-9) there remains a lack of consensus (10,11). While many clinical trials have reported a hypocholesterolemic effect of garlic (12-16), others have found no detectable lipid response (17-20). The lack of concordance may be due to the substantial heterogeneity in the design and conduct of these trials. Many of the garlic trials reported have been limited or flawed. For example, in a meta-analysis conducted by Warshafsky et al. 28 trials were identified (8). Of these 28 trials, 21 were excluded for having inadequate study designs, or for including a substantial proportion of subjects with normal cholesterol concentrations. Another possible reason for the inconsistency has been the type of garlic preparation used, which has included fresh garlic (21,22), steam-distilled

garlic oil (17), aged garlic extract (13), and dehydrated powdered garlic (14,19,23).

Possible confounders in these studies were often unreported, including adherence level or changes in weight, diet or exercise. Other important differences among these trials include the dosage, duration and characteristics of subjects studied. Although widely promoted commercially for its cholesterol-lowering ability, the benefit of garlic supplementation in lipid management remains uncertain.

We conducted a double-blind, parallel treatment, randomized clinical trial of garlic supplementation in a group of moderately hypercholesterolemic adults (LDL-C 130 to 190 mg/dL). Adherence, weight, diet and exercise were monitored throughout the study to insure their constancy. Plasma lipids were measured every 2-weeks to examine the possible time-dependent nature of any effects. This study's purpose was to determine if 12-weeks of garlic supplementation would lower plasma LDL-cholesterol concentrations in moderately hypercholesterolemic adults, and if so, to examine a possible dose response using 0, 500 and 1,000 mg/day of powdered garlic. Blood pressure and other plasma lipids were examined as secondary outcome measures.

SUBJECTS AND METHODS

Sample

Participants were recruited from the general public and from Stanford University employees, primarily through newspaper advertisements. Men and women who were 30-65 years of age, with fasting plasma LDL-cholesterol 130-190 mg/dL, fasting plasma triacylglycerol < 300 mg/dL, and a body mass index (BMI) between 19-31 kg/m² were

invited to enroll. Exclusions were pregnant women, smokers, prevalent heart disease, diabetes or use of lipid or blood pressure lowering medications within the past month. Following a telephone screening interview, 158 were eligible for cholesterol testing; of these 60 were found to have LDL-cholesterol and triacylglycerol concentrations in the eligibility range. Of those eligible, 7 chose not to participate and 53 were randomly assigned to the three groups. This investigation was reviewed and approved by the Stanford University Human Subjects Committee. All participants signed an informed consent form prior to enrollment.

Study Design

The study was a randomized, double-blind, placebo-controlled, 12-week parallel treatment, clinical trial. Participant enrollment was from June to October, 1997. Participants were scheduled for nine clinic visits following random assignment to one of three treatment groups (Figure 1).

Figure 1 about here

Materials and Supplements

Garlic and placebo tablets (Nutrilite Division of Amway Corporation, Buena Vista, CA) were provided in sealed bottles that each contained a two-week supply (42 tablets/container). Full-dose garlic tablets contained 333 mg garlic powder, and the half-dose tablets 167 mg. The three full dose garlic tablets were equivalent to approximately 1.5 cloves of fresh garlic. Tablets were to be taken three times daily with meals. The garlic tablets also contained modest amounts of dried watercress, parsley and licorice: 33.

33 and 21 mg, respectively, in the full-dose tablets, and half these amounts in the half-dose tablets. The placebo was composed of a dextrose-cellulose base. All tablets were formulated to be identical in appearance. In addition, each bottle of tablets included a cotton filler infused with a drop of garlic oil that imparted a strong odor to disguise the identity of the tablet composition. Each set of six bottles for an individual participant was labeled with a unique code number to identify its contents. Study staff and participants were blinded to the treatment assignment.

Data Collection

At every clinic visit, weight and blood pressure were recorded and ≥ 12 -hour fasting blood samples were collected by venipuncture in EDTA tubes. Adherence was monitored by tablet count. Every two weeks during clinic visits the study tablet bottles were turned in, the number of tablets that had not been consumed in that period were recorded, and a new bottle with a two-week supply was dispensed. In addition, participants were asked to report their level of adherence in an end-of-study questionnaire.

Participants were advised to maintain their weight, as well as their usual diet and activity levels for the study's duration. When weight deviated by more than 1 kg from baseline, they were counseled to alter their energy intake accordingly. Food intake was assessed at baseline, mid-study and end-study, using three-day food records. No restrictions were placed on participants' intake of fresh garlic in order to avoid other dietary changes that may have limited the generalizability of the findings.

Exercise and physical activity behaviors were assessed using a modified version of the Paffenbarger Physical Activity Questionnaire (1978) (24). A physical activity score for the previous week was determined by quantifying and combining the number of hours of both moderate and vigorous activity, in both work and leisure settings. The questionnaires were administered at baseline, mid-study and at the end of study.

Effectiveness of the blinding and the tolerance of the study tablets were assessed at the end of the study by a self-administered questionnaire. Upon the study's completion, participants were asked if they knew to which of the three treatments they were assigned, or if they "could not tell." They were also asked to indicate whether they had experienced any of twelve gastrointestinal symptoms during the study, and the frequency of each symptom (never, seldom, occasionally, often, frequently or always).

Fasting blood samples were collected by venipuncture in EDTA tubes, refrigerated immediately and centrifuged within 2 hours. Plasma samples were stored in Wheaton vials and frozen at -70°C until a given participant had completed the protocol, at which time a set of samples for that participant were thawed and analyzed in a single batch to minimize laboratory variability. Plasma concentrations of total cholesterol and triacylglycerol (with subtraction of a free glycerol blank) were measured by enzymatic procedures using established methods of the Lipid Research Clinics (ABA 200 instrument, Abbot Laboratories) (25,26). HDL-C was measured by dextran sulfate-magnesium precipitation (27) followed by enzymatic determination of cholesterol (25). LDL-cholesterol was calculated according to the method of Friedewald (28) unless the triacylglycerols were $>400\text{ mg/dL}$, in which case the LDL-C value was considered

missing data. The lipoprotein laboratory assays as monitored by the Lipid Standardization Program of the Centers for Disease Control and Prevention (Atlanta, GA) were consistently within specified limits. Blood pressure was measured manually by use of a sphygmomanometer on the right arm while participants were seated after at least a 5-minute rest. Three blood pressure readings were taken at approximately two-minute intervals and the average of the second and third readings were used.

Data analyses

Nutrient analyses were conducted using Food Processor, Version 7.0 (ESHA, Salem, OR). Statistical tests were performed using the SAS computer program (SAS Institute Inc. Cary, NC). Descriptive statistics are presented for the baseline characteristics of the participants. The baseline characteristics and study hypothesis were tested using analysis of variance (ANOVA) and two separate approaches. The first approach was to test for between-group differences in plasma lipids or blood pressure using repeated measures ANOVA and the average of two baseline and two end-study measurements. A general linear model (GLM) was used to include pre-post changes in saturated fat and weight as covariates. A second approach was to use the time-series of nine assessments for each participant to calculate the slope of the response for total, LDL and HDL cholesterol and triacylglycerols. These data were then used to test for between-group differences in slopes in a GLM using ANOVA with adjustment for baseline lipid concentration as a covariate. All statistical tests were two-tailed using a significance level (α) of 0.05.

RESULTS

Table 1 shows the three groups were similar at baseline in age, weight, diet composition, plasma lipids and blood pressure. Fifty-one participants completed the study. Two participants dropped out in the first week because of unforeseen scheduling conflicts, one each from the half-dose and full-dose groups.

Table 1 about here

Minimal side effects were reported. Of the twelve symptoms enumerated in the questionnaire, participants reported seldom to occasional incidents of belching, body odor, stomach gas, flatulence and constipation. Eleven, 47 and 24 percent of the placebo, half-dose and full dose group, respectively, reported at least one of these gastrointestinal symptoms. However, the symptoms were mild in severity and did not impair participation or adherence. The blinding technique proved to be effective. The study tablets were indistinguishable by sight, taste and smell. Twenty-eight percent of the placebo group, 27% of the half-dose and 0% of the full-dose group correctly guessed their assigned dose.

Weight, dietary composition, physical activity and adherence remained constant throughout the study. The differences between groups for these potential confounders were modest and not statistically significant. The average difference from baseline in weight was less than 1 kg for each group at each clinic visit, and at end-study the differences from baseline (mean $\pm$ SD) were 0.4 ± 1.6 , 0.0 ± 1.6 and 0.8 ± 1.6 kg for placebo, half-dose and full-dose, respectively. The change from baseline in % energy

from saturated fat was determined at both mid-study and end-study to be: $+0.1 \pm 3.0\%$ and $+0.8 \pm 3.6\%$ respectively, for placebo (n=15 with complete data); $+1.8 \pm 3.7\%$ and $+2.6 \pm 3.1\%$ for half-dose (n=16 with complete data); and $+1.8 \pm 2.4\%$ and $+1.4\% \pm 3.5\%$ for full-dose (n=14 with complete data). The change from baseline in physical activity score at mid-study and end-study was: -2.6 ± 14.3 hours and $+2.3 \pm 8.4$ hours, respectively, for placebo; -5.8 ± 16.8 hours and -5.3 ± 12.0 hours for half-dose; and $+0.5 \pm 11.0$ hours and $+1.9 \pm 7.8$ hours for full-dose (complete data for all but placebo with n=1 missing). Adherence as assessed separately by pill count and by a self-administered questionnaire was $\geq 92\%$ across all treatment groups.

Plasma lipid changes are presented in Figure 2. Mean changes in total-cholesterol were $+1.4\%$, $+1.4\%$ and -4.0% for the placebo, half-dose and full-dose groups, respectively. Corresponding changes in LDL-C concentrations were 0.0% , $+0.9\%$ and -6.1% ; changes in HDL-C concentrations were $+5.6\%$, $+4.5\%$ and $+4.6\%$; and changes in triacylglycerol concentrations were $+1.4\%$, -2.5% and -5.9% . Although the full-dose group had the most favorable lipid changes in 12-weeks of intervention, none of the between group differences was statistically significant.

Figure 2 about here

Using the nine blood samples for each participant, a second statistical approach was used to test for differences in the slopes of the 12-week changes. Again, no statistically significant differences in lipid changes were detected. For placebo, half-dose and full-dose, respectively, the slopes (mg/dL/day) were - Total-C: -0.04 ± 0.39 , 0.03 ± 0.28 and -0.11 ± 0.32 ; LDL-C: -0.02 ± 0.27 , 0.03 ± 0.25 and -0.07 ± 0.41 ; HDL-C: 0.02

± 0.07 , 0.03 ± 0.06 and 0.03 ± 0.08 ; and triacylglycerols: -0.14 ± 0.52 , 0.03 ± 0.44 and -0.10 ± 0.42 . Results of the 0, 2, 4, 6, 8, 10 and 12-week plasma lipid assessments are presented in Table 2. There were no significant between-group differences in blood pressure during the study in this normotensive population (data not presented).

Table 2 about here

DISCUSSION

In this study, 12-weeks of daily supplementation with 1,000 mg of garlic powder in tablet form had a marginal and non-significant lowering effect on LDL-cholesterol concentrations (-6.1%) in a group of moderately hypercholesterolemic adults. LDL-C concentrations in the placebo and half-dose groups remained virtually unchanged from baseline. There were also no significant pre-post differences observed among the three groups for total-C, HDL-C, triacylglycerols or blood pressure, although in absolute numbers, the full-dose group had the largest decrease in total-C and triacylglycerols.

The marginal and non-significant LDL-cholesterol lowering effect reported in this study lends itself to several possible interpretations. In the strictest sense, the wide range of within-group variability in LDL-C changes over the 12-weeks made the groups statistically indistinguishable. The range of LDL-C changes was -46 to +38 mg/dL in the placebo group, -31 to +34 mg/dL in the half-dose group, and, -62 to +24 mg/dL in the full-dose group. Furthermore, the absolute decrease in LDL-C concentrations of 10.1 mg/dL (6.1%) in the full-dose group relative to the placebo group (0.0 mg/dL change) would be considered clinically inadequate as a single therapy for most individuals trying

to control elevated LDL-C concentrations in the 130-190 mg/dL range. On the other hand, the effect of garlic supplementation may be additive or synergistic with hygienic (e.g. high-fiber diets, weight loss, exercise) and pharmacological lipid management approaches, and could prove to be a modestly useful adjunct to other therapies not tested here. In addition, if every 1% decrease in plasma cholesterol is associated with a 2%-3% decrease in relative risk for coronary heart disease (1), then the absolute change in LDL-C reported here, though statistically non-significant, could have public health relevance at the population level. However, from a public health perspective, another important consideration would be that we have reported findings from a supervised, closely monitored, short-term, efficacy study. In a free-living population, adherence to a regimen of 3 tablets, taken daily, with three meals, over periods longer than the 12-week protocol of our study, would likely be less than was achieved here. In this regard, it is important to note that the half-dose of garlic supplementation used in this study had no effect, both statistically and in terms of the absolute average LDL-C concentration change.

The findings are consistent with the existing literature. The 95% confidence intervals around the plasma lipid concentration changes in the full-dose treatment arm would include the main effects of most of the clinical trials that have been published to date. The conclusions from two meta-analyses of clinical trials were that plasma lipid lowering therapy with various forms of garlic supplementation resulted in a 9-12% reduction in plasma cholesterol (7,8). Steiner et al (1996) (13) used an unrealistically large dose of a specific garlic preparation, nine tablets per day of aged-garlic extract, and reported only a 4.6% lowering of plasma LDL-C concentrations. In their discussion they

suggested that the previous meta-analyses may have overestimated the effect of garlic, for reasons such as publication bias for positive findings. Considerable variability exists even in well designed studies. For example, Adler et al (1996) (12), using a commercial powdered garlic tablet, reported a significant net drop of 13.1% in LDL-C concentrations relative to the placebo group in 12-weeks, and Jain et al (1993) (14), using the same product and a similar design, reported a significant net decrease of 8% in LDL-C concentrations in moderately hypercholesterolemic adults. In contrast, a recent study reported no effect of steamed garlic oil supplementation on cholesterol concentrations in hypercholesterolemic adults after 12-weeks (17). One explanation could be that the oil is not as effective as garlic powder (29). However, three other studies (18,19,23), using the same dosage of the same commercial product as the studies above (12,14), reported no significant effect. Some of the discrepancies reported in these studies can be explained by the heterogeneity that exists among them in terms of study design, duration, subject characteristics, adherence, or confounders such as weight, diet, and exercise. Taken as a whole, this current body of evidence suggests that the hypocholesterolemic effect of garlic intake, when taken in the form of supplementation, is marginal to negligible, similar to the findings in this study.

The possibility that a significant cholesterol-lowering effect of garlic supplementation was missed in the current study might be raised if there were strong mechanistic data to support this. Chi et al (1982) suggested that garlic supplemented diets may decrease the activities of lipogenic enzymes and enhance the excretion of neutral steroids and bile acids as was observed in a rat study (30). Other studies using rat

hepatocytes have demonstrated that garlic supplemented diets resulted in a dose-dependent inhibition of cholesterol biosynthesis at different enzymatic steps (31-34). Despite the reported inhibition in these studies, a limitation of *in vitro* and animal models is that the results may not be physiologically relevant *in vivo* in humans. Further work on a possible mechanistic link between garlic compounds and plasma lipid metabolism is warranted.

A recent review by Lawson (1998) adds another important perspective regarding the discrepancies in the literature described above (35). It raises the possibility that a true hypocholesterolemic effect of garlic supplements could go undetected if the *in vivo* production of allicin from alliin was inefficient and inconsistent. Lawson points out that raw garlic contains both alliin and alliinase but no allicin. Upon crushing or chewing the allinase quickly converts the alliin to allicin. Allicin is stable in the digestive tract and would be available for absorption under these circumstances. The alliin in garlic tablets, on the other hand, would not be converted to allicin until the tablet appears in the upper gastrointestinal tract and is digested. Lawson suggests that the digestive process might inactivate the alliinase before it is allowed to complete the conversion of alliin to allicin. If this were the case, then the yield of bioavailable allicin in garlic supplements would vary, and could be lower than expected. To test this, Lawson conducted disintegration tests exposing garlic tablets to standardized, simulated gastrointestinal conditions and reported substantial variability in the “effective allicin yield” from various formulations. Tablets that were enterically coated generated the highest effective allicin yield suggesting that avoidance of the stomach's acidic environment might be needed to allow

the conversion of alliin to allicin. It appears that standardization and documentation of both total allicin yield and “effective allicin yield” in future supplement studies is warranted. This also points to the importance of conducting further trials with fresh garlic, despite the limitation that such studies could not easily be blinded.

There were several strengths of this investigation. The bimonthly clinic visits promoted adherence and allowed for multiple blood sampling. The use of multiple serial blood samples added sensitivity to the plasma lipid analyses. The documentation of weight, diet and exercise stability, and the high adherence suggest that consistent garlic tablet consumption was achieved and that this study was shielded from important confounding variables. The inclusion of a simultaneous placebo group in the trial further supports the adequacy of the trial for testing the hypothesis that garlic supplementation lowers plasma LDL-cholesterol. Finally, to our knowledge, the inclusion of a half-dose in the design was an approach that had not been used before, which allowed us to examine possible intermediate dose or threshold effects, which were not observed.

This trial was also subject to several limitations. Garlic intake from food sources was not controlled during the study. Participants were asked to refrain from consuming large quantities of garlic, but were also told they should maintain their habitual diets and did not have to abstain completely from garlic in their food while on-study. It was felt this approach made the results more generalizable, although differences in garlic intake between the study groups could have confounded the results. Random assignment and a low-drop out rate should have reduced this concern. Another limitation was the small sample size that had limited statistical power to detect a difference in the order of the

magnitude that was found. A larger sample size might have detected a statistical significance, but an LDL-C change of <10% in an efficacy trial is of borderline clinical significance. Another issue concerned the formulation of the supplement. First, the garlic tablet was a botanical blend that included licorice and small amounts of flavoring agents including watercress and parsley. One study has reported a hypocholesterolemic effect of licorice in albino rats (36). Although data regarding the hypolipidemic effect in humans is unavailable, if true the licorice may have partially influenced the results of the study (i.e. the modest, non-significant LDL-C lowering in the full-dose group would only be partially attributable to the garlic). Also the garlic supplements used in this study were not standardized for allicin content as other studies have done (12,14,19,23). If it is allicin and its metabolites that are the hypocholesterolemic agents, and if the garlic supplement used in this trial had a lower than normal content of these compounds, the potential cholesterol lowering effects in this study may have been attenuated. However, the powdered garlic tablets were prepared in a single lot for this study using standardized procedures, and the investigation was completed in a timely fashion, so we have no reason to believe the active ingredients used were impaired. Finally, this trial focused on two doses, of a single product, in a specified population over a defined time-period of 12-weeks, and the effects on plasma lipids. This leaves many questions unanswered regarding the effect of garlic intake, in various forms, in different dosages, on a wide range of other human health factors, such as immune function, coagulation and vasodilation (5).

Implications

The present data does not support the use of garlic supplementation alone, at the doses currently used in commercially available tablets, to substantially lower LDL-cholesterol concentrations in moderately hypercholesterolemic adults. However, this does not preclude the possibility that garlic intake may contribute to an overall lipid lowering effect of an approach that includes other hygienic or pharmacological therapies. It is also important to consider the food choices that accompany raw or cooked garlic intake. For example, vegetable fiber in a stir-fry containing garlic and plant-based foods, or saturated fat from butter on garlic bread would have opposite effects on plasma lipids, regardless of the effect or lack of effect of the garlic. Finally, increased garlic intake, ingested in its various forms, may confer other health outcomes or benefits not measured in this study.

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Table 1. Baseline characteristics of study population (mean $\pm$ SD)

	Supplementation Group		
	Placebo	Half-Dose	Full-Dose
Males/Females (n)	9/9	7/10	10/6
Age (years)	51.6 $\pm$ 8.1	53.4 $\pm$ 8.9	50.2 $\pm$ 8.3
Weight (kg)	77.8 $\pm$ 10.2	76.9 $\pm$ 17.7	76.3 $\pm$ 12.1
Body mass index (kg/m ²)	26.9 $\pm$ 2.8	26.0 $\pm$ 4.2	25.4 $\pm$ 3.6
Dietary Composition[†]			
Total Energy/day (Kcal)	1960 $\pm$ 490	1840 $\pm$ 375	2075 $\pm$ 600
Total Fat (% Energy)	26.6 $\pm$ 8.6	26.4 $\pm$ 7.4	27.0 $\pm$ 7.5
Saturated Fat (% Energy)	8.5 $\pm$ 4.3	8.4 $\pm$ 3.1	8.1 $\pm$ 3.1
Dietary Fiber (gm/1000 Kcal)	11.3 $\pm$ 6.1	11.6 $\pm$ 3.7	11.9 $\pm$ 4.4
Alcohol (% Energy)	2.2 $\pm$ 3.1	4.7 $\pm$ 4.8	2.6 $\pm$ 2.9
Physical Activity Score [‡]	19.9 $\pm$ 15.7	25.0 $\pm$ 23.1	22.2 $\pm$ 18.2
Outcome Measures			
Total-C (mg/dL)	230 $\pm$ 24	231 $\pm$ 21	235 $\pm$ 27
LDL-C (mg/dL)	158 $\pm$ 20	151 $\pm$ 18	163 $\pm$ 18
HDL-C (mg/dL)	46 $\pm$ 10	54 $\pm$ 18	46 $\pm$ 12
Triacylglycerol (mg/dL)	133 $\pm$ 48	125 $\pm$ 63	126 $\pm$ 56
Blood Pressure (mmHg)			
Systolic	117.0 $\pm$ 11.9	115.0 $\pm$ 11.0	118.1 $\pm$ 14.5
Diastolic	75.8 $\pm$ 7.6	74.9 $\pm$ 7.8	79.1 $\pm$ 7.3

[†] Does not include participants with missing baseline or end-study food records (placebo n=3, half-dose n=1, full-dose n=1)

[‡] Includes combined hours per week of moderate & intense physical activity combined as defined in methods.

None of the between-group differences was statistically significant at baseline.

Table 2. Plasma lipid measures (mean $\pm$ SD) over 12 weeks

	Base- line	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12	Baseline to 12-wk (p-value)
<i>mg/dL</i>								
Total- Cholesterol								
	230 $\pm$ 24	226 $\pm$ 27	223 $\pm$ 19	227 $\pm$ 23	227 $\pm$ 26	228 $\pm$ 23	233 $\pm$ 28	0.3
Placebo								
	231 $\pm$ 21	235 $\pm$ 27	232 $\pm$ 19	239 $\pm$ 23	233 $\pm$ 23	235 $\pm$ 26	234 $\pm$ 26	
Half-Dose								
	235 $\pm$ 27	232 $\pm$ 29	232 $\pm$ 29	235 $\pm$ 18	230 $\pm$ 18	228 $\pm$ 24	225 $\pm$ 23	
Full-Dose								
LDL- Cholesterol								
	158 $\pm$ 20	160 $\pm$ 21	149 $\pm$ 19	152 $\pm$ 17	153 $\pm$ 26	156 $\pm$ 19	158 $\pm$ 25	0.5
Placebo								
	151 $\pm$ 18	153 $\pm$ 23	151 $\pm$ 15	158 $\pm$ 18	152 $\pm$ 18	149 $\pm$ 19	153 $\pm$ 20	
Half-Dose								
	163 $\pm$ 18	160 $\pm$ 21	158 $\pm$ 12	159 $\pm$ 20	155 $\pm$ 17	154 $\pm$ 18	153 $\pm$ 22	
Full-Dose								
HDL- Cholesterol								
	46 $\pm$ 10	45 $\pm$ 12	46 $\pm$ 12	46 $\pm$ 12	47 $\pm$ 12	47 $\pm$ 12	48 $\pm$ 12	0.9
Placebo								
	54 $\pm$ 18	53 $\pm$ 16	54 $\pm$ 16	55 $\pm$ 17	55 $\pm$ 16	58 $\pm$ 15	56 $\pm$ 18	
Half-Dose								
	46 $\pm$ $\pm$ 12	46 $\pm$ $\pm$ 12	46 $\pm$ $\pm$ 14	50 $\pm$ 13	51 $\pm$ 11	49 $\pm$ 14	49 $\pm$ 14	
Full-Dose								
Triacylglycerol								
	133 $\pm$ 48	158 $\pm$ 70	140 $\pm$ 57	145 $\pm$ 52	134 $\pm$ 49	126 $\pm$ 53	135 $\pm$ 39	0.7
Placebo								
	125 $\pm$ 63	142 $\pm$ 72	131 $\pm$ 59	129 $\pm$ 69	132 $\pm$ 69	137 $\pm$ 69	122 $\pm$ 64	
Half-Dose								
	126 $\pm$ 56	135 $\pm$ 65	122 $\pm$ 48	133 $\pm$ 71	118 $\pm$ 51	129 $\pm$ 58	119 $\pm$ 52	
Full-Dose

Figure 1. Study Design

Figure 2. Mean $\pm$ SEM 12-week changes in plasma lipids. Analysis of covariance detected no significance between-group differences.

CHAPTER 3: SUMMARY AND RECOMMENDATIONS

Summary

A double-blind, randomized, placebo-controlled, parallel treatment clinical trial was conducted to study the effect of garlic supplementation in modestly hypercholesterolemic adults. Fifty-one adults were randomized to receive tablets containing 1000 mg/d garlic powder at full-dose, 500 mg/d (half-dose) or placebo for 12 weeks. At every bimonthly visit plasma lipids and blood pressure were measured and participants received a two-week supply of the tablets. Adherence, dietary intake, physical activity and weight remained comparable in the three treatment arms during the intervention. Although in absolute numbers the full-dose group had the most favorable overall changes in lipid parameters, the decrease of 6.1% in the primary outcome LDL-C in this group, was statistically not significantly different from the half-dose or placebo groups. No significant differences were observed for the secondary outcome measures including other plasma lipids and blood pressure. A dose-response was not evident using two levels of supplementation. Garlic powder tablets were not efficacious in substantially lowering LDL-cholesterol in this group of modestly hypercholesterolemic adults.

Recommendations for Future Research

Garlic continues to merit strong interest as much more than a fashionable supplement. In this study blood lipids were investigated and were reported to have marginal effects. However, research in garlic therapy needs to focus on its plethora of

implied health benefits, the vehicle of administering garlic and effects of cultivation factors.

It is important for researchers to recognize the lacunae in data pertaining to the best delivery vehicle, effective dosage of garlic supplements and the availability of standardized formulations. The ancient belief of the beneficial effect of garlic on the cardiovascular system such as blood pressure lowering, enhanced immune function, improved coagulation factors and anti-oxidant capacity were beyond the scope of this investigation and need to be clinically assessed

Food scientists have developed methodologies to successfully identify and quantify the thermally labile active sulfur compounds in garlic extracts. The ratio of these compounds varies with growing conditions and other controlled variables (fertilization, irrigation, humidity, plant varieties and storage). Research is warranted to evaluate the changes in active compounds for well-defined garlic cultivates. The development of products containing bioactive constituents of garlic appears to be a significant determinant of its pharmacological activity.

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APPENDICES

APPENDIX A

Effect of Garlic Supplementation on Plasma Lipids in Hypercholesterolemic Adults

Experimental Subject's Bill of Rights

Persons who participate in a medical experiment are entitled to certain rights. These rights include but are not limited to the subject's right to:

- be informed of the nature and purpose of the experiment;
- be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be utilized;
- be given a description of any attendant discomforts and risks reasonably to be expected;
- be given an explanation of any benefits to the subject reasonably to be expected, if applicable;
- be given a disclosure of any appropriate alternatives, drugs, or devices that might be advantageous to the subject, their risks and benefits;
- be informed of the avenues of medical treatment, if any, available to the subject after the experiment, if complications should arise;
- be given an opportunity to ask any questions concerning the experiment or the procedures involved;
- be instructed that consent to participate in the medical experiment may be withdrawn at any time and the subject may discontinue participation without prejudice;
- be given a copy of the signed and dated consent form; and
- be given the opportunity to decide to consent or not to consent to a medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion or undue influence on the subject's decision.

Are you currently participating in any other research studies? ☐ yes ☐ no

Overview of study

You have been identified as a possible participant in a nutritional study of the effect of garlic supplements on blood cholesterol levels among adults with moderately elevated LDL-cholesterol (130 - 190 mg/dL). In the study we will examine whether garlic supplements, taken three times each day for 12 weeks, will affect blood cholesterol levels. We will also provide garlic supplements in more than one dose in order to determine if the change in blood cholesterol levels is proportional to the amount of garlic supplementation. Some of the study participants, a subset, will be invited to take part in an additional part of the study that involves examining the health of an artery in the arm (brachial artery). We hope to learn whether garlic supplements can have a favorable impact on plasma LDL-cholesterol levels, and on other indicators of health in the blood circulation system.

You were identified as a possible participant through an initial telephone interview. During the telephone interview it was determined that you:

- are between the ages of 30 and 65 years,
- are not currently taking any medications for blood pressure or blood cholesterol.

- are not a smoker,
- may have a moderately elevated blood level of LDL-cholesterol (the "bad" type).
- would be willing to take three tablets each day for 12 weeks,
- are available to come to Stanford University campus every two weeks to pick up a supply of supplements and return empty supplement containers from the previous two weeks.
- are in general good health.

Identifying participants with modestly elevated plasma LDL-cholesterol

In order to be eligible for the study, your LDL-cholesterol must be between 130 and 190 mg/dL (mild to moderate elevation). We will test this for you, and you will receive a copy of your results at no cost. If your LDL-cholesterol level is between 130 and 190 mg/dL you will be invited to enroll in the study.

If your LDL-cholesterol is lower than 130 mg/dL, you will not be invited to enroll in the study. If your LDL-cholesterol is higher than 190 mg/dL, you will be advised to bring these results to the attention of your personal physician.

For those who do enroll in the study, the various procedures and activities are described below.

Specific procedures and activities:

Cholesterol Screening Blood Draw

The initial cholesterol screening described above involves an overnight fast (12 hours) and a brief visit to the clinic at 730 Welch road on the Stanford campus for a blood draw that should take only a few minutes.

Introductory meeting

This one-time meeting will last for 1-hour. During this meeting we will show you the facilities at 730 Welch road where the study will be conducted. You will also be given the chance to have any of your questions answered. In addition, you will be given instructions how to complete a 3-day food record which will be used to analyze your intake of various nutrients.

Blood draw

Blood will be drawn, at the 730 Welch road facility, by trained personnel who are certified in Basic Life Support, using standard procedures. Blood drawing will be done on nine different occasions over the 12-week study period (twice at the start of the study, then once every two weeks (weeks #2, 4, 6, 8 & 10), and then twice at the conclusion of the study). For each blood draw you will be expected to abstain from all foods and beverages for the previous 12-hours. For this reason we will schedule all of your blood draws in the early morning, and ask you to fast from the prior evening until the blood draw. The amount of blood drawn will be either 20 mL (7 times), or 70 mL (twice). Since there are 15 mL in a tablespoon, the two amounts of blood to be drawn are equivalent to approximately 1 1/3 tablespoons (20 mL), or 4 2/3 tablespoons (70 mL). The

entire amount, 280 mL, is approximately half the amount of a typical blood donation at a blood bank (1 pint). Since the blood drawing is spread out over a 12-week period this amount of blood loss would not be considered to pose any significant health consequences.

The blood samples will be used to measure the following:

Cholesterol (Total, LDL [the "bad" type] and HDL [the "good" type])

Triglyceride (a measure of the level of fat in your blood)

Monocyte adhesiveness (an indicator of an early stage in the progression of deposits of cholesterol in arteries, known as atherosclerosis)

Physical exam

On the same day as your first blood drawing for the study, a brief physical exam will be performed by a certified Physician's Assistant to establish your general health. The exam will involve the following measures:

1. A review of your medical history.
2. Weight - using a standard balance beam scale. This measurement will be taken while you are wearing only light clothing and without shoes.
3. Height - using a standard height scale.
4. Blood Pressure - using standard procedures while you are resting comfortably.

Estimate of total time required, length and frequency of procedures

The full study will involve your participation for a total of 12 weeks. The estimated time involved in participation in each procedure or activity is outlined below.

Introductory meeting - (ONE HOUR, ONCE, just prior to your enrollment in the study)

This one-time meeting will last for 1-hour. Here you will be given instructions how to fill out two questionnaires. First, a 3-day food record which will be used to analyze your intake of various nutrients. Second, an activity record which will be used to determine your usual pattern of physical activity.

Starting date physical examination - (30 MINUTES, ONCE, prior to receiving supplements)

Once you have attended the Introductory meeting and decided to enroll, you will be scheduled for a starting date. On this date you will come in for a physical examination to determine your general health, and then a fasting blood sample will be taken. At this point you will be given your first packet of supplements.

Daily supplements - (A FEW MINUTES, EACH DAY)

Each day you will be expected to consume three study tablets. It is expected you will take them at different times during the day, preferably with meals in the morning, at lunch, and in the evening. It should take you no more than a few minutes to do this each day.

Biweekly weight checks and packet pick-ups - (5 MINUTES PER VISIT, 10 VISITS)

Once every two weeks, for a total of 5 times, you will be expected to come to our facility at 730 Welch road on the Stanford campus to be weighed, to pick-up a two week supply of supplements, and to drop-off the empty packets of supplements consumed during the previous two weeks.

Diet assessment (3-day records) - (10-15 MINUTES/DAY, 9 DAYS TOTAL)

On three separate occasions (week #0, #4 and #12), you will be expected to keep a 3-day

food record. Each time, the three days will be a combination of 2 weekdays and 1 weekend day. You will be instructed on how to keep a pencil and paper record of every item you eat or drink for a particular day, including specific amounts consumed and specific preparation methods used.

Activity recall - (10-15 MINUTES, 3 TIMES)

On three separate occasions (week #0, #4 and #12), you will be asked to complete a 7-day activity recall questionnaire. The questionnaire will be administered by an interviewer either in person or over the phone. You will be asked to recall how many hours during the previous seven days you spent sleeping, engaged in strenuous physical activity, and engaged in moderate physical activity.

Risks and benefits associated with clinic procedures

The risks associated with the above clinic procedures are minimal. The most substantial risk anticipated is the minimal risk of bruising, light-headedness, or possible infection during blood drawing as described earlier. The amounts and type of garlic supplements used in this study have previously been given to a wide range of individuals and has been well tolerated. The ultrasound assessment of endothelial function described earlier can be uncomfortable due to the inflation of a blood pressure cuff on your arm which is maintained for five minutes, but no risks are anticipated with this procedure.

Benefits for participants include the free assessment of blood cholesterol levels on several occasions. You will also learn about the composition of your diet and how to improve it.

Participants generally find this personal information very interesting and usually enjoy the experience undergoing these tests. **WE CANNOT AND DO NOT GUARANTEE OR PROMISE THAT YOU WILL RECEIVE ANY BENEFITS FROM THIS STUDY.**

The investigators are eager that all participants in the study regard themselves as partners in research and will ensure that those taking part are fully informed of results obtained at the end of the program. Results of your individual medical tests will be returned to you, and (by request) to your private physician. Any data that may be published in scientific journals will not reveal the identity of the subjects. Patient information may be provided to Federal and regulatory agencies as required. The Food and Drug Administration, for instance, may inspect research records and learn your identity if this study falls within its jurisdiction.

Financial payments and costs for participants

No payment will be provided to participants for this project. Nutrilite Inc., a division of Amway Corp., is the primary institute providing financial support for this study. All costs for the study will be supported by the research grant and there will be no additional costs to you for participation in this study, other than transportation to our facility.

Other research projects being conducted at the same time as this one

While participating in this study, you should not take part in any other research projects without approval from all of the investigators. This is to protect you from possible injury arising from such things as extra blood drawing, interactions of research drugs, or similar hazards. Your decision whether or not to participate will not prejudice you or your

medical care. If you decide to participate, you are free to withdraw your consent and discontinue participation at any time without prejudice to you and without effect on your medical care. You will be told if any new information is learned which may affect your condition or influence your willingness to continue participation in this study.

If you have any questions, we expect you to ask us. If you have any additional questions later, Dr. John W. Farquhar, MD at (415) 723-6052 will be happy to answer them. At the discretion of the principal investigator, subjects may be taken out of this study.

All forms of medical diagnosis and treatment -- whether routine or experimental -- involve some risk of injury. In spite of all precautions, you might develop medical complications from participating in this study. If such complications arise, the researchers will assist you in obtaining appropriate medical treatment, but this study does not provide financial assistance for additional medical or other costs. You do not waive any liability rights for personal injury by signing this form. For further information, please call (415) 723-5244 or write the Administrative Panel on Human Subjects in Medical Research, Administrative Panels Office, Stanford University, Stanford, CA 94305-5532. In addition, if you are not satisfied with the manner in which this study is being conducted or if you have any questions concerning your rights as a study participant, please contact the Human Subjects Office at the same address and telephone number.

YOUR SIGNATURE INDICATES THAT YOU HAVE READ AND UNDERSTAND THE ABOVE INFORMATION, THAT YOU HAVE DISCUSSED THIS STUDY WITH THE PRINCIPAL INVESTIGATOR AND HIS STAFF, THAT YOU HAVE DECIDED TO PARTICIPATE BASED ON THE INFORMATION PROVIDED, AND THAT A COPY OF THIS FORM HAS BEEN GIVEN TO YOU.

Print Name

Signature

Date

Signature of Investigator or Witness

Date

Approval Date 7/1/97

Expiration Date 6/2/98

APPENDIX B

GARLIC & CHOLESTEROL STUDY

Telephone Screening Questionnaire

Date of initial contact _____	Phone message ____ (Y/N & date)
Date of phone screen _____	Updated excel list ____ (Y/N)
I) Included based on phone screen ____ (Y/N) [if yes continue below with IV]	
II) Excluded based on phone screen: ____ (Y/N) Reason: _____	
OK to keep name/address for referral to future studies: ____ (Y/N)	
III) Undetermined based on phone screen: ____ (Y/N) Reason: _____	
IV) Blood screening date/time: _____ Rescheduled date if no show: _____	
Called night before to confirm appointment and remind about fast: ____ (Y/N)	
Sent package: ____ (Y/N)	Date sent: _____ Included consent form: ____ (Y/N)
V) Called about results of screen: ____ (Y/N) Date: ____ Left message: ____ (Y/N)	
Mailed results: ____ (Y/N) Date: _____	
VI) After blood screening: Included: ____ (Y/N) Excluded: ____ (Y/N) _____	
Notes: _____	

Name: _____

Address _____

(Street, City & Zip Code)

Phone: (home) () _____ Time: _____ (work) () _____ Time: _____

e-mail: _____ Fax/pager: _____

Thank you for your interest in participating in our Stanford study on Garlic supplements.

Is this a good time to ask you a few questions that will determine whether you may be eligible to be a study participant?

If yes- All of your responses to the following questions will be kept strictly confidential.

After you have answered these questions you may ask me any questions that you have.

If no- What time will be convenient to call you back? _____

What would you estimate your current weight to be? Weight _____ lb

What would you estimate your current height to be? Height _____ in

BMI: _____ kg/m²

Yes _____ No _____ Are you between 30 and 65 years of age?

Date of birth: _____

Yes _____ No _____ Are you currently taking any cholesterol lowering medications?

If yes, name of medications _____

Yes _____ No _____ Are you taking any blood pressure lowering medications?

If yes, name of medications _____

Yes _____ No _____ Are you taking any medications on a regular basis?

If yes, name of medications _____

Yes _____ No _____ Are you taking any garlic supplements?

Yes _____ No _____ Do you happen to know your cholesterol level from a recent test?

If yes, result _____

Yes _____ No _____ Are you a smoker?

How much alcohol do you drink on an average/week? _____

Yes ____ No ____ Are you pregnant or lactating? (for women only)

Yes ____ No ____ Have you ever been diagnosed with diabetes?

Do you have any health concerns that might affect your participation? _____

Yes ____ No ____ Are you currently enrolled as a participant in any other study?

Yes ____ No ____ Will you be living/working in the area for at least 3 months?

Yes ____ No ____ Do you have a vacation planned in the next 3 months that is longer than two weeks?

If yes, date/duration _____

Yes ____ No ____ Would you be willing to take 3 tablets of supplements each day for 12 weeks?

Yes ____ No ____ I want to make you aware that this is a randomized, double-blinded and controlled study, so that neither you nor us will know if you will be taking a garlic or a placebo supplement. Would this knowledge in any way affect your participation?

How are you planning to get to our center?

__ Car __ Public transportation __ Bike __ Other

Finally, for my records, could you tell me where you heard about the study?

_____ Posted flyer

_____ Advertisement: Where? _____

_____ Announcement in the mail

_____ email

_____ Family member/friend/acquaintance Name; _____

_____ Other

If not eligible

I am sorry, but one of our criterion for participation is _____ and based on your answer to that question you do not meet this criterion. Thank you very much for your interest. We conduct many studies here on the Stanford campus, and in the future you may be eligible for other studies. Are you interested in being contacted for other studies? Yes ____
No _____

If eligible

Based on all your answers to this set of questions, it appears that you may be eligible to be a participant in our study. The next step in determining your eligibility is for us to screen your cholesterol level. We will make an appointment for you to come to Stanford and have your blood drawn in our laboratory. We will also send you a consent form in the mail that you can fill out at home and return to us before your first blood screening. The consent form will allow us to determine your cholesterol level with a blood draw. If your LDL- cholesterol is between 130-190mg/dl we will invite you to join one of our group- introductory meetings and complete a pre-study questionnaire. This is when we ask you to make a decision about your participation in our study. Do you have any more questions about the study?

APPENDIX C

GARLIC STUDY
Pre-Study Questionnaire

Name: _____ Date: _____

Address: _____
(Street, City and Zip Code)

Day Phone: _____ Evening Phone: _____

1. What is your age? _____ Birthdate: ____/____/____
month day year2. What is your current marital status?
☐ Single ☐ Widowed
☐ Married ☐ Divorced/Separated3. What is your race or ethnic background?
☐ White, not of Hispanic origin ☐ Asian
☐ Black, not of Hispanic origin ☐ Pacific Islander
☐ Hispanic ☐ American Indian/Alaskan native
☐ Other (please specify) _____

4. What is the total number of years of schooling you have completed? (circle one number)

Grade School
1_2_3_4_5_6_7_8_High School
1_2_3_4_College
1_2_3_4_5_6+_

5. What is your current primary occupation? _____

If retired, prior primary occupation _____

6. Compared to others your age, would you say your health in general is...
☐ Excellent ☐ Fair
☐ Very Good ☐ Poor
☐ Good

7. Are you currently taking any of the following more than once a week?

If yes, please tell us how often.

	Yes	No	If yes, identify
How often?			
• Nutritional supplements and/or vitamins or minerals	☐	☐	_____

(including multivitamin)			
• Diet pills, appetite suppressant, insulin, estrogen or other	☐	☐	_____

female hormones, any other medication.			_____

_____			_____
_____			_____

8. Have you ever been told by a doctor or health professional that you have or have had any of the following:

	Yes	No	If yes, please say what and when
• Arthritis/Joint disorders	☐	☐	_____
• Cancer	☐	☐	_____
• Diabetes	☐	☐	_____
• Digestive problems	☐	☐	_____
• Food Allergy/ Intolerance	☐	☐	_____
• Gynecological disorders	☐	☐	_____
• Heart problems	☐	☐	_____
• High blood pressure	☐	☐	_____
• Kidney/bladder disorders	☐	☐	_____
• Liver problems	☐	☐	_____
• Lung problems	☐	☐	_____
• Osteoporosis	☐	☐	_____
• Low blood sugar (hypoglycemia)	☐	☐	_____
• Stomach problems	☐	☐	_____
• Other	☐	☐	_____

9. The following questions are about your current eating habits. Please check the response that best describes your usual behavior as it is now.

During the week, how often do you...

	6-7 days	3-5 days	1-2 days	Never
a. Eat breakfast?	☐	☐	☐	☐
b. Eat lunch?	☐	☐	☐	☐
c. Eat dinner?	☐	☐	☐	☐
d. Eat in between meals or after evening meals?	☐	☐	☐	☐

APPENDIX D

GALC Physical Activity Record

Name _____

- How many flights of stairs do you usually climb up each day?
Number of flights _____ Let 1 flight = 10 steps
- How many city blocks or their equivalent do you regularly walk each day?
Number of blocks _____ Let 12 blocks = 1 mile
- List any sports or recreation you have participated in during the past WEEK. Please include only the time you were physically active (i.e. actual time in jogging, bicycling, swimming, brisk walking, gardening, carpentry, calisthenics, etc.).

Sport, recreation or other physical activity	Number of TIMES in a WEEK	Avg time per EPISODE	
		Hours	Minutes
1.			
2.			
3.			
4.			
5.			

- List any OTHER sports or recreation you have actively participated in during the past MONTH. Please remember seasonal sports or events.

Sport, recreation or other physical activity	Number of WEEKS per MONTH	Avg time per WEEK when active	
		Hours	Minutes
1.			
2.			
3.			
4.			
5.			

- On a usual weekday and a weekend day, how much time do you spend on the following activities? Total for each day should add up to 24 hours.

	Usual weekday Hours per day	Usual weekend day Hours per day
a. Vigorous activity (e.g. digging in the garden, strenuous sports, jogging, chopping wood, sustained swimming, brisk walking, heavy carpentry, bicycling on hills)		
b. Moderate activity (e.g. housework, light sports, regular walking, golf, yard work, lawn mowing, painting, repairing, light carpentry, dancing, bicycling on level ground)		
c. Light activity (e.g. office work, driving a car, strolling, personal care, standing with a little motion)		
d. Sitting activity (e.g. eating, reading, desk work, watching TV, listening to the radio)		
e. Sleeping or reclining		
	Total=24 Hrs	Total=24 Hrs

THANK YOU

APPENDIX E

Food Diary

Name: _____

Date: _____

For three days, write down everything you eat or drink, *excluding* water. Use the blank recording sheets provided in this package and bring your completed 3-day food record to your individual appointment.

Make sure you include one weekend day and 2 weekdays. Please use days that are representative of your typical eating habits. Try not to let recording interfere with your usual eating pattern.

Give us as much detail as you can about the food you eat, especially portion size and preparation methods. Information from food labels for unusual products will be helpful to us - feel free to actually attach the labels themselves.

Thank you very much for your participation.

Use this space to write down any unusual recipes used or other any other information you think might be helpful to us:

STANFORD UNIVERSITY
Stanford, California 94305-5532

ROBERT MARCUS, M.D.
 CHAIR, PANEL ON MEDICAL HUMAN SUBJECTS
 c/o Administrative Panels Office

(415) 723-5244
 (415) 725-8013 (fax)

CERTIFICATION OF HUMAN SUBJECTS APPROVAL

DATE: July 1, 1997

TO: J.W. Farguhar, M.D.
 W.L. Haskell, Ph.D. and C. Gardner, Ph.D.
 Department of Med/SCMDP

FROM: Chairman, Administrative Panel
 on Human Subjects in Medical Research

PROTOCOL ENTITLED:
REVISION: Effect of Garlic Supplement on Plasma Cholesterol in
 Hypercholesterolemic Adults.

The Panel approved human subject involvement in your research project on July 1, 1997.

The expiration date of this approval is June 2, 1998. If this project is to continue beyond that date, please submit an updated proposal in advance for the Panel's re-approval. If this proposal is used in conjunction with any other human experimentation or if it is modified in any way, it must be re-approved for these special circumstances. In addition, the Panel requests prompt notification of any complications which may occur during any experimental procedure.

All continuing projects and activities must be reviewed and re-approved at least annually by the Panel. Panel approval of any project is for a maximum period of one year. It is the responsibility of the investigator to resubmit the project to the committee for annual review.

 for Robert Marcus, M.D., Chairman

Funding Agency: (Amway Corporation pend.) (SP08 18733)
Period of Time: 07/01/97-06/02/98
Investigational New Drugs: N,
Investigational New Device: N,
Cooperating Institution: N
Assurance Number: M1272
IRB #01

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Monterey Park Northridge Pomona
Sacramento San Bernardino San Diego
San Francisco San Jose San Luis Obispo
San Marcos Stanislaus Sutter

TO: Lorraine Chatterjee
21281 Glenmont Dr.
Saratoga, CA 95070

FROM: Nabil Ibrahim,
Acting AVP, Graduate Studies & Research

DATE: June 4, 1999

The Human Subjects-Institutional Review Board has approved
your request to use human subjects in the study entitled:

**"Effect of Garlic Supplementation on Plasma
Lipids in Hypercholesterolemic Adults"**

This approval is contingent upon the subjects participating in your research project being appropriately protected from risk. This includes the protection of the anonymity of the subjects' identity when they participate in your research project, and with regard to any and all data that may be collected from the subjects. The Board's approval includes continued monitoring of your research by the Board to assure that the subjects are being adequately and properly protected from such risks. If at any time a subject becomes injured or complains of injury, you must notify Nabil Ibrahim, Ph.D., immediately. Injury includes but is not limited to bodily harm, psychological trauma and release of potentially damaging personal information.

Please also be advised that all subjects need to be fully informed and aware that their participation in your research project is voluntary, and that he or she may withdraw from the project at any time. Further, a subject's participation, refusal to participate, or withdrawal will not affect any services the subject is receiving or will receive at the institution in which the research is being conducted.

If you have any questions, please contact me at
(408) 924-2480.