Garlic Supplementation and Lipoprotein Oxidation Susceptibility

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Interventions which make serum lipoproteins less susceptible to oxidation may be antiatherogenic. The antioxidant properties of garlic which have been demonstrated *in vitro* led us to investigate the effects of garlic supplements on lipoprotein oxidation susceptibility in humans. Ten healthy volunteers were given 600 mg/d of garlic powder (6 tablets of Kwai®) for two weeks in a placebo-controlled, randomized, double-blind crossover trial. We found that although serum lipid and lipoprotein levels were not lowered in this short time period, the *ex vivo* susceptibility of apolipoprotein B-containing lipoproteins to oxidation was significantly decreased (−34%). Because garlic has been reported to beneficially affect serum lipid levels, platelet function, fibrinolysis and blood pressure, this additional effect of retarding lipoprotein oxidation may contribute to the potential antiatherosclerotic effect of garlic.
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There is growing evidence that the oxidation of low density lipoproteins (LDL) may play a significant role in the development of atherosclerosis (for a review, see Ref. 1). Accordingly, interventions which can prevent the oxidation of LDL may be expected to slow atherogenesis, reducing the incidence of coronary heart disease, possibly even without lowering serum LDL levels.

There are currently three nutrients (ascorbic acid, α-tocopherol, monounsaturated fatty acids, Refs. 2–4), one food preservative (butylated hydroxytoluene, Ref. 5) and one drug (probucol, Ref. 6) that function as *in vivo* antioxidants. The latter two have also been shown to be antiatherosclerotic in animal models (5,6). Garlic is a food which has has been reported to lower cholesterol levels (7), and in addition, to have a beneficial effect on a number of other physiological disorders contributing to heightened cardiovascular risk, *e.g.,* high blood pressure (8), enhanced platelet aggregation (9–11), delayed fibrinolysis (9) and vasoconstriction (9). A recent report suggested that garlic powder extracts also act as antioxidants *in vitro* (12). Therefore, we conducted a trial in humans to determine the effects of short-term garlic supplementation on plasma lipid levels and on lipoprotein oxidation susceptibility (LOS).

MATERIALS AND METHODS

Ten healthy, normolipidemic subjects, five males and five females (mean age 32 ± 10 years) volunteered for the study. The subjects were taking no medications known to affect serum lipids, and none were taking garlic or significant quantities of antioxidants (vitamins C or E or β-carotene) in their background diets. Subjects were asked to keep their smoking and exercise patterns constant throughout the study. Informed consent was obtained from each subject prior to beginning the study, which had been approved by the Human Subjects Committee of the University of Kansas Medical Center (Kansas City, KS).

The subjects were instructed to take six 100-mg tablets of garlic powder a day for two weeks (Kwai®, Lichtwer Pharma GmbH, Berlin, Germany) or placebo. The study had a randomized, placebo controlled, double-blind crossover design. There was a one-week washout period between the two treatment periods.

Maintaining dietary stability was highly stressed throughout the study, and subjects were advised to avoid any foods containing significant amounts of garlic. Diets were monitored by having the subjects complete two three-day diet diaries (one during each treatment period) which were analyzed by Professional Nutrition Systems, Inc. (Kansas City, KS). This was done to determine whether the changes in plasma lipid levels, if any, were due to the garlic or to a change in diet. Results of these analyses were expressed as the RISCC rating (ratio of ingested saturated fat and cholesterol to calories) which summarizes the major cholesterol raising nutrients in the diet as a single numerical score. An RISCC rating of 20–24 is typical of the American diet (13).

Blood samples were drawn (always after a 12-h overnight fast) at the beginning and the end of each test period. Lipids and lipoproteins in plasma containing ethylenediaminetetracetic acid (1 mg/mL) were analyzed for cholesterol and triglycerides using enzymatic methods on a Cobas Mira (Roche Diagnostics, Belleville, NJ). The plasma high density lipoprotein (HDL) cholesterol levels were measured following precipitation of the apoprotein B (apoB)-containing lipoproteins with heparin/manganese chloride. LDL cholesterol was estimated by the Friedwald equation (14). These methods have been described in detail elsewhere (15), and were carried out in our laboratory which participates in the Lipid Standardization Program of the Centers for Disease Control (16).

LDL phospholipid and cholesteryl ester fatty acid composition. Lipids were extracted from LDL isolated from plasma by sequential ultracentrifugation between densities 1.019 and 1.063 kg/L. Thin-layer chromatography was used to separate the various lipid classes, and the cholesteryl ester and phospholipid bands were obtained. The lipids were transmethylated with boron trifluoride and analyzed by gas-liquid chromatography in a GC9A Gas Chromatograph (Shimadzu Corp., Columbia, MD), equipped with a 30-m, 0.32 mm i.d., SP2330 capillary column. These methods were described in detail previously (15).

LOS test. A 500-μL plasma sample was treated with 50 μL of a solution containing 0.2 mM dextran sulfate (MW 50,000; Genzyme, Cambridge, MA) and 0.5 M $MgCl_2 \cdot 6H_2O$ to precipitate the apoB-containing lipoproteins [LDL and very low density lipoproteins (VLDL)]

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Abbreviations: apoB, apolipoprotein B-100; HDL, high density lipoproteins; LDL, low density lipoproteins; LOS, lipoprotein oxidation susceptibility; MDA, malondialdehyde; RISCC, ratio of ingested saturated fat and cholesterol to calories; TBA, thiobarbituric acid; TBARS, TBA reactive substances; TCA, trichloroacetic acid; VLDL, very low density lipoproteins.

according to Bachorik and Albers (17). After centrifugation at 3,000 rpm at 20°C for 10 min, the supernatant was removed, and 1 mL of 6% bovine serum albumin and another 50 μL of the dextran sulfate magnesium solution was added. The solution was briefly vortexed and recentrifuged as above to wash away any HDL or residual serum proteins (except, of course, albumin). The supernatant was removed and the washed precipitate (containing LDL and VLDL) was dissolved in 2.5 mL of 4% NaCl. A volume of redissolved precipitate containing 100 μg of non-HDL cholesterol was combined with sufficient 4% NaCl to give a total volume of 500 μL (approximately a 1:5 dilution). Fifty μL of a 0.5 mM $CuCl_2 \cdot 2H_2O$ solution was added (final copper concentration was 46 μM), and then the samples were incubated at 37°C in a shaking water bath for 3 h. Next, thiobarbituric acid reactive substances (TBARS) were measured (18) by adding 2 mL of the TBARS reagent to each tube. [This reagent contained 26 mM thiobarbituric acid (TBA) and 0.92 M trichloroacetic acid (TCA) in 0.25 N HCl. The TBA was added first, heated and stirred. After it was dissolved, the TCA was added, and the solution brought to volume with 0.25 N HCl. The reagent was stored in a dark bottle at room temperature.] The mixture was heated at 100°C in a waterbath for 15 min. After removing and cooling the tubes, 2.5 mL *n*-butanol was added, the tubes were vortexed, and then centrifuged for 15 min at 3,000 rpm at room temperature. The pink upper layer was removed and the optical density was determined in a spectrophotometer at 532 nm. A standard curve was constructed with malondialdehyde (MDA, 0.5–16 nmol/mL), and the results were expressed as nmol of MDA produced per mg of non-HDL cholesterol. The coefficient of variation of the method was 4% intraassay and 9% interassay. The mean LOS value for 20 normal subjects was 66 ± 22 nmol MDA/mg non-HDL cholesterol. Using this assay, we have shown that probucol treatment reduced LOS by 95%, while fish oil supplementation (high in polyunsaturated fatty acids) raised LOS by 45% (Harris, W.S., unpublished data).

Statistical evaluation. As the subjects served as their own control for four observations (pre and post, garlic and placebo), the data were analyzed by ANOVA with repeated measures followed by the Neuman-Kuels *post-hoc* test. A P value of <0.05 was required for statistical significance.

RESULTS AND DISCUSSION

Two weeks of garlic supplementation did not alter plasma total, LDL or HDL, cholesterol or triglyceride levels (Table 1). This was not a wholly unexpected finding as previous investigators had reported only small decreases in lipid levels after 4 wks, with maximum effects noted at 16 wks (7). Thus, longer periods of supplementation would probably be needed to change these parameters.

On the other hand, garlic supplementation significantly reduced the susceptibility of the apoB-containing lipoproteins to copper-induced oxidation (Fig. 1). LOS values went from 80 ± 25 to 53 ± 32 nmol MDA/mg non-HDL cholesterol, a 34% decrease ($P < 0.05$). Values after two weeks of placebo were unchanged at 72 ± 19 *vs.* 73 ± 16 nmol MDA/mg non-HDL cholesterol. Only about half of the subjects actually experienced decreases in LOS, and two showed remarkable reductions. The relative non-responsiveness of the other half of the subjects suggests that other factors (dose, duration, endogenous antioxidants) may modulate the effectiveness of garlic.

Dietary stability was confirmed in this study by analysis of diet diaries during both phases. RISCC ratings were identical (23 ± 5) in both phases, indicating that the subjects were consuming a typical American diet, and that there was no change in background diets between periods which would be expected to alter plasma lipid levels. Because the diets were stable, the effects observed on LOS were likely to be due to the garlic powder supplements.

A change in LDL fatty acid composition (*i.e.*, a reduced amount of polyunsaturated fatty acids) could have been responsible for the reduced susceptibility of lipoproteins to oxidative stress during the garlic period. However, we found no change whatever in LDL phospholipid or cholesteryl ester fatty acid patterns (Table 2). Data on LDL vitamin E levels are not available, but because garlic powder contains no vitamin E, it seems unlikely that changes in α-tocopherol status would explain these findings.

Kourounakis and Rekka (12) recently tested the antioxidant properties of garlic powder, Kwai and alliin (the odorless precursor which is converted to odoriferous allicin when exposed to alliinase, an enzyme released when the

TABLE 1

Effects of Two Weeks of Kwai® Supplementation on Serum Lipids and Lipoproteins in Ten Healthy Volunteers (mg/dL)[a]

	Placebo		Garlic	
	Before	After	Before	After
Total cholesterol	174 ± 39	173 ± 38	176 ± 37	175 ± 39
Triglyceride	75 ± 22	78 ± 19	79 ± 21	79 ± 24
HDL-cholesterol	54 ± 7	55 ± 7	56 ± 6	56 ± 8
LDL-cholesterol	104 ± 36	103 ± 33	106 ± 34	104 ± 36

[a]HDL, high density lipoprotein; LDL, low density lipoprotein.

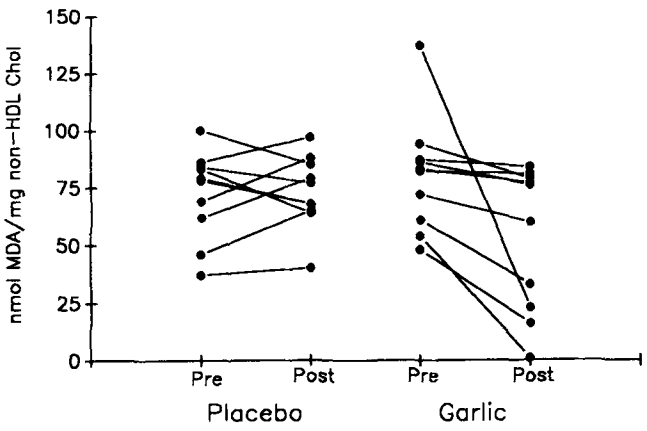

FIG. 1. Six garlic powder tablets (Kwai®) or placebo were given daily to ten healthy volunteers for two weeks in a randomized, crossover design. Lipoprotein oxidation susceptibility (LOS) was assessed (see Materials and Methods) pre and post dosing for both treatments. Individual results are presented here; mean LOS was reduced by 34% by Kwai treatment and increased by 1% by placebo ($P < 0.05$). HDL, high density lipoprotein; MDA, malondialdehyde.

TABLE 2

Saturated, Monounsaturated and Polyunsaturated Fatty Acid Composition of Low Density Lipoproteins Cholesteryl Esters and Phospholipids During Placebo and Garlic Phases (mol% of total fatty acids)[a]

	Phospholipids			Cholesteryl esters		
Period	Sat	Mono	Poly	Sat	Mono	Poly
Placebo	70 ± 5	7 ± 1	23 ± 5	26 ± 8	16 ± 3	58 ± 8
Garlic	68 ± 5	7 ± 2	24 ± 5	26 ± 11	16 ± 6	57 ± 8

[a]Sat, saturated; mono, monounsaturated; poly, polyunsaturated.

garlic clove is crushed). They reported that both the garlic powder and Kwai were effective antioxidants when tested against rat hepatic microsomes stressed by ascorbic acid/Fe^{2+} and evaluated by TBARS generation. The garlic powder and alliin were also tested for their ability to scavenge hydroxyl radicals. Only the powder was effective at concentrations between 0.8 and 3.3 mM.

The application of these results to the *in vivo* situation is unclear, at best, as blood levels of garlic powder constituents (alliin or allicin) in subjects taking the products have eluded quantitation. Garlic is rich in a variety of sulfur-containing compounds (diallyldisulfide, vinyldithiines, ajoene), which are all potential antioxidants, but finding the putative *in vivo* antioxidant may be difficult.

In view of these considerations, it was with some skepticism that we evaluated the antioxidant effects of Kwai in healthy volunteers, and it was also with some surprise that we discovered that some component of this garlic preparation appeared to retain its antioxidant effect *in vivo*, even protecting isolated lipoproteins from oxidative stress. Future studies will be needed to determine exactly which component is responsible.

We conclude that 600 mg of Kwai taken for only two weeks significantly decreased the lipoprotein oxidation susceptibility without altering serum cholesterol levels in normal adults. If confirmed, such an effect may be considered to be potentially antiatherogenic. Trials should be undertaken to examine this effect in larger groups, for longer periods of supplementation, and in patients at increased risk for heart disease. In addition, the effects of garlic on atherosclerosis-prone animal models should be evaluated.

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REFERENCES

1. Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C., and Witztum, J.L. (1989) *N. Engl. J. Med. 320,* 915-924.
2. Jialal, I., Vega, G.L., and Grundy, S.M. (1990) *Atherosclerosis 82,* 185-191.
3. Esterbauer, H., Dieber-Rotheneder, M., Striegl, G., and Waeg, G. (1991) *Am. J. Clin. Nutr. 53,* 314S-321S.
4. Parthasarathy, S., Khoo, J.C., Miller, E., Barnett, J., Witztum, J.L., and Steinberg, D. (1990) *Proc. Natl. Acad. Sci. USA. 87,* 3894-3898.
5. Björkhem, I., Henriksson-Freyschuss, A., Breuer, O., Diczfalusy, U., Berglund, L., and Henriksson, P. (1991) *Arterio. Thromb. 11,* 15-22.
6. Carew, T.E., Schwenke, D., and Steinberg, D. (1987) *Proc. Natl. Acad. Sci. USA. 84,* 7725-7729.
7. Mader, F.H. (1990) *Arzneim Forsch/Drug Res. 40,* 3-8.
8. Auer, W., Eiber, A., Hertkorn, E., Hoehfeld, E., Koehrle, U., and Lorenz, A. (1990) *Brit. J. Clin. Pharmacol. 44 (Suppl 69),* 3-6.
9. Kiesewetter, H., Jung, F., Pindur, G., Jung, E.M., Mrowietz, C., and Wenzel, E. (1991) *Intl. J. Clin. Pharmacol. Therap. Toxicol. 29,* 151-155.
10. Makheja, A.N., Vanderhoek, J.Y., and Bailey, J.M. (1979) *Lancet 1,* 781.
11. Harenberg, J., Giese, C., and Zimmerman, R. (1988) *Atherosclerosis 74,* 247-249.
12. Kourounakis, P.N., and Rekka, E.A. (1991) *Res. Comm. Chem. Path. Pharmacol. 74,* 249-252.
13. Harris, W.S., Held, S.J., and Dujovne, C.A. (1988) *Arteriosclerosis 8,* 633a.
14. Friedwald, W.T., Levy, R.I., and Fredrickson, D.S. (1972) *Clin. Chem. 19,* 499-502.
15. Harris, W.S., Dujovne, C.A., Zucker, M., and Johnson, B. (1988) *Ann. Intern. Med. 109,* 465-470.
16. Myers, G.L., Cooper, G.R., Winn, C.L., and Smith, S.J. (1989) *Clinics in Lab. Med. 9,* 105-135.
17. Bachorik, P.S., and Albers, J.J. (1986) *Methods Enzymol. 129,* 78-100.
18. Buege, J.A., and Aust, S.D. (1978) *Methods Enzymol. 52,* 302-310.

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