A randomized factorial study of the effects of long-term garlic and micronutrient supplementation and of 2-wk antibiotic treatment for *Helicobacter pylori* infection on serum cholesterol and lipoproteins[1−3]

Lian Zhang, Mitchell H Gail, Yu-que Wang, Linda Morris Brown, Kai-feng Pan, Jun-ling Ma, Harunobu Amagase, Wei-cheng You, and Roxana Moslehi

ABSTRACT

Background: Little is known about the long-term effects of garlic or micronutrient supplementation on total, HDL, and LDL cholesterol in disease-free persons.

Objective: We aimed to assess the effects of long-term supplementation with garlic and micronutrients and of short-term amoxicillin and omeprazole treatment on serum total, HDL, and LDL cholesterol in a rural Chinese population.

Design: We conducted a randomized, double-blind, placebo-controlled, 2 × 2 × 2 and 2 × 2 factorial study of precancerous gastric lesions in 3411 subjects in Linqu County, Shandong Province, China. Thirty-four subjects were randomly selected from each of 12 treatment strata. Sera were analyzed at 3.3 and 7.3 y to measure effects on total, HDL, and LDL cholesterol after 2-wk twice-daily treatment with 1 g amoxicillin and 20 mg omeprazole and supplementation throughout the study with *1*) 2 capsules twice daily, each containing 200 mg aged garlic extract and 1 mg steam-distilled garlic oil, or *2*) twice-daily micronutrient capsules containing 250 mg vitamin C, 100 IU vitamin E, and 37.5 mg selenium.

Results: Regressions adjusted for covariates indicated increases of 0.22 mmol total cholesterol/L (*P* = 0.01) and 0.19 mmol LDL/L (*P* = 0.02) after 7.3 y of micronutrient supplementation, but no effect of garlic supplementation or short-term amoxicillin and omeprazole treatment.

Conclusions: In this rural Chinese population with low meat intake and moderate cholesterol concentrations, long-term garlic supplementation had no effect on lipid profiles, whereas micronutrient supplementation was associated with small but significant increases in total and LDL-cholesterol concentrations at 7.3 y.   *Am J Clin Nutr* 2006;84:912–9.

INTRODUCTION

High concentrations of total and LDL cholesterol and low concentrations of HDL cholesterol are risk factors for cardiovascular disease (CVD) (1). Garlic or garlic extracts have been recommended for treatment or prevention (or both) of CVD (2), as well as for other hyperlipidemia-related disorders (3). Some controlled trials indicate that garlic reduces total cholesterol and serum triacylglycerols and elevates HDL concentrations. However, most such studies were small and of short duration and were confined to patients with hypercholesterolemia or CVD (4). A recent 12-wk study of 75 normolipidemic subjects did not find an effect of garlic powder on total, LDL, or HDL cholesterol (5), which leaves much uncertainty as to its effects in the general population. Moreover, few controlled trials have been conducted on the effects of long-term supplementation with vitamins and selenium on lipid profiles, and the data on prevention of CVD are conflicting (*see* Discussion).

In 1995, the National Cancer Institute (NCI) and the Beijing Institute for Cancer Research (BICR) initiated a randomized, double-blind, placebo-controlled factorial trial, the Shandong Intervention Trial (SIT), to ascertain whether intervention with 3 treatments, alone or in combination, would lead to a reduction in the prevalence of advanced precancerous gastric lesions and gastric cancer. Those treatments were amoxicillin and omeprazole [only in subjects with *Helicobacter pylori* (HP) infection], dietary supplementation with vitamins E and C and selenium, and dietary supplementation with steam-distilled garlic oil and ethanol-aqueous garlic extract. A list was obtained of all persons aged 35–64 y (*n* = 4010) residing in 13 villages selected at random from 4 townships in Linqu County, Shandong Province, China. Of these 4010 persons, 3599 (90%) underwent endoscopy in 1994 and were invited to participate in the SIT (**Figure 1**).
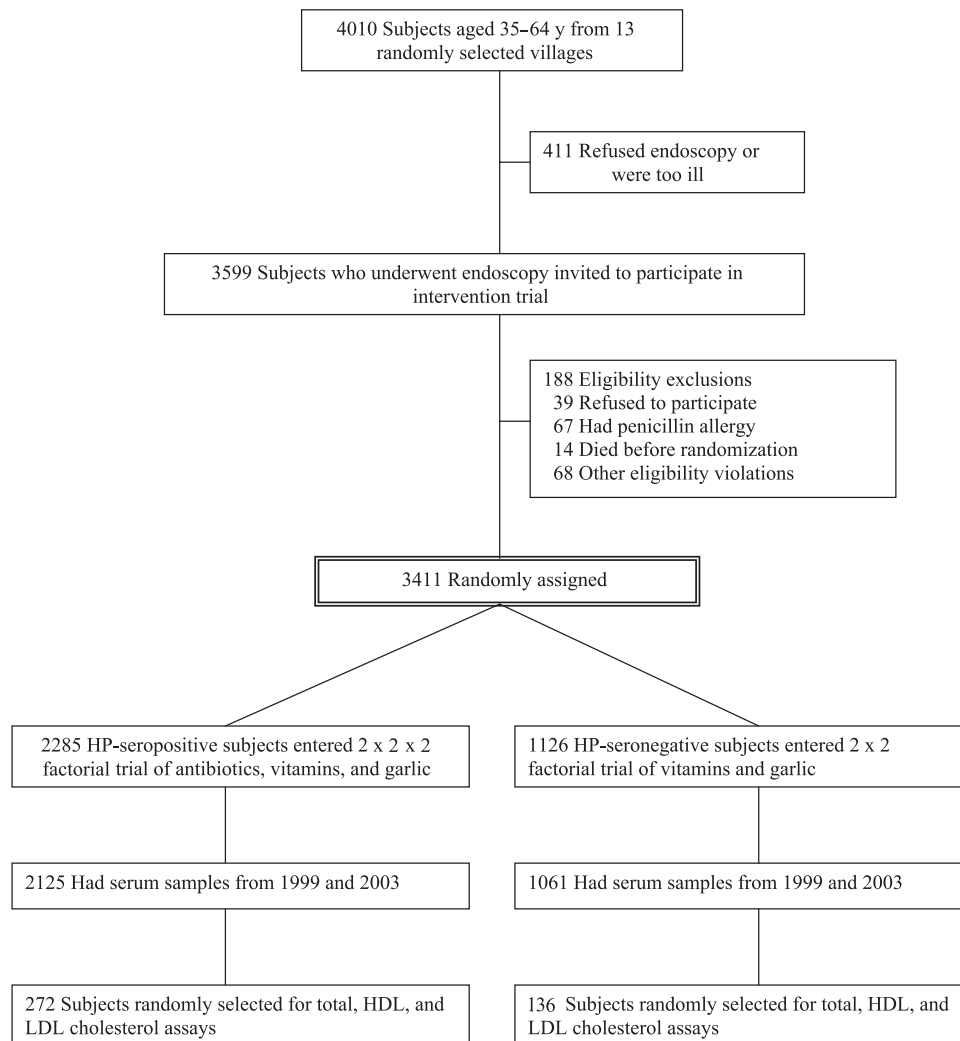
FIGURE 1. Participant flow chart. HP, *Helicobacter pylori*. The 272 HP-seropositive subjects were selected in groups of 34 from each of the 8 HP treatment strata, and the 136 HP-seronegative subjects were selected in groups of 34 from each of the 4 HP-seronegative treatment strata.

Subjects were excluded from randomization if they lived outside the study area, were outside the 35–64 y age range, were missing baseline HP serologic tests, refused to participate, were allergic to penicillin, had a previous diagnosis of cancer (except non-melanoma skin cancer), or had died before randomization, as described previously in detail (6). Thus the study population of 3411 subjects was representative of the general population in the area.

Although the SIT was designed to study precancerous gastric lesions, the current study was conducted to ascertain whether the administration of garlic or vitamin and selenium supplements (or both) for 7.3 y (88 mo) affected serum concentrations of total, HDL, or LDL cholesterol in the general population in this region of rural China. For this purpose, we used a stratified random sample from the 12 treatment strata of the SIT. Secondary objectives were to examine the effect of a one-time, 2-wk course of HP treatment on these lipid profiles and the effects of the 3 interventions on all-cause and CVD mortality.

SUBJECTS AND METHODS

Study population and sampling design

The SIT randomized study population ($n = 3411$) fell into 12 treatment strata, corresponding to a $2 \times 2 \times 2$ factorial design in subjects who were seropositive for HP at baseline in 1994 and a 2×2 factorial design for those who were seronegative for HP at baseline (**Table 1**). For the study of serum lipids, we subsampled the 3186 participants for whom serum samples from 1999 were available to obtain 408 subjects; 34 of these subjects were randomly selected from each of the 12 treatment strata (Figure 1 and Table 1). Coded sample identification was used so that neither the technicians who retrieved the samples nor the personnel who performed the laboratory analyses knew which treatments had been assigned. We analyzed total, LDL, and HDL cholesterol in all 408 subjects for whom serum samples from 1999 were available and in 381 of these subjects for whom serum samples from 2003 were available.

TABLE 1
Participants by intervention[1]

	Amoxicillin and omeprazole	Garlic supplement	Vitamin and selenium supplement	Randomly assigned	Eligible with serum samples in 1999	Sampled
				n	*n*	*n*
HP-seropositive at baseline						
	A	A	A	286	261	34
	A	A	P	285	262	34
	A	P	A	286	269	34
	A	P	P	285	268	34
	P	A	A	285	267	34
	P	A	P	286	268	34
	P	P	A	286	266	34
	P	P	P	286	264	34
HP-seronegative at baseline						
	P	A	A	282	263	34
	P	A	P	281	265	34
	P	P	A	281	268	34
	P	P	P	282	265	34
Totals				3411	3186	408

[1] A, active treatment; P, placebo; HP, *Helicobacter pylori.*

Treatments

Amoxicillin and omeprazole or the corresponding placebo were given for a 2-wk period between September and November 1995 (treatment was staggered by village), and garlic or vitamin and selenium supplements or their placebos were given for a period of 88 mo from 30 November 1995 to 31 March 2003. The 3 interventions were 1 g amoxicillin and 20 mg omeprazole, both twice daily, for 2 wk (both: Astra, East Asia Region, Singapore); 2 capsules twice daily, each containing 200 mg aged garlic extract (Kyolic; Wakunaga of America Co, Ltd, Mission Viejo, CA) and 1 mg steam-distilled garlic oil; and twice-daily vitamin and selenium supplements containing 250 mg vitamin C, 100 IU vitamin E (α-tocopherol), and 37.5 μg selenium (all: Sino-American Shanghai Squibb Pharmaceuticals Ltd, Shanghai, China). Placebos for each of these interventions were given when the active intervention was not assigned. The doses of vitamins and selenium were chosen to modestly exceed the doses that, in a previous intervention trial in Henan Province, China (7), showed a protective effect against gastric cancer mortality. The median daily intake of garlic in Linqu County was 3.6 g. The daily dose of 800 mg aged garlic extract (AGE) is roughly equivalent to the dry weight of 8 g garlic, or ≈2.2 times the median daily intake in Linqu County. The dose of 4 mg steam-distilled garlic oil, which in our preparation provides ≈4 mg allyl polysulfides, is roughly equivalent to the amount of allyl polysulfides that can be obtained from 4 g cooked garlic, or slightly above the daily consumption in Linqu County.

The compliance of the participants was monitored through monthly counting of unconsumed pills left in the bottles and by quarterly measurements of serum concentrations of *S*-allylcysteine (a component of the aged garlic extract), vitamin C, and α-tocopherol in sera from 80 randomly sampled participants. For logistical reasons, the garlic and vitamin and selenium supplements were not given in June and July 1999, and the garlic supplements were not given in September 2002 (8, 9). The subjects given amoxicillin and omeprazole who had continued evidence of HP infection according to a urea breath test (*n* = 386) were given an additional 2-wk course of antibiotic treatment in May 1996 (6).

Measurement of total, HDL, and LDL cholesterol

During physical examinations in 1999 and 2003, 5 mL blood was collected from each fasting participant in SIT. The blood specimen was obtained in the morning and was allowed to clot in the dark at room temperature for 30 min and then centrifuged at $1000 \times g$ at room temperature for 15 min. One aliquot of serum was stored immediately at -20 °C and then moved within 2 or 3 d into a -70 °C freezer. The serum concentrations of total, HDL, and LDL cholesterol were measured in the Clinical Laboratory of Beijing Cancer Hospital. Total cholesterol was tested by a cholesterol oxidase-phenol aminophenazone (CHOD-PAP) method with a cholesterol kit (Randox Laboratories Ltd, Crumlin, United Kingdom). HDL and LDL cholesterol were tested by using standard methods with direct HDL- and LDL-cholesterol kits (Randox Laboratories, Ltd), respectively, and all measurements were made with a Hitachi 7170 analyzer (Tokyo, Japan) (10).

Quality control was performed by using routine laboratory procedures for assays of total, HDL, and LDL cholesterol. A duplicate test was performed for each sample measurement. If the difference between the 2 values exceeded 5%, a repeat assay was performed to confirm the results. Standard samples with high or low concentrations of total, HDL, and LDL cholesterol were analyzed daily to ensure that the assay procedure was within quality-control limits and to ensure that the equipment was properly calibrated. The interassay CVs of total, HDL, and LDL cholesterol were 4.20%, 3.02%, and 2.91%, respectively.

Statistical analysis

Analyses were performed on an intention-to-treat basis (ie, all randomly assigned participants were analyzed in the treatment group to which they were assigned at randomization), regardless of the subject's degree of compliance. Two-sided values of $P <$ 0.05 were used for all analyses. Analyses were performed by

using either SAS (version 8.2; SAS Institute Inc, Cary, NC) or SPSS (version 13.0; SPSS Inc, Chicago, IL) statistical software.

Descriptive statistics were calculated to compare the mean cholesterol and lipoprotein concentrations between the subjects who received garlic and those who did not receive garlic. A similar descriptive analysis was performed for vitamin and selenium supplementation and for amoxicillin and omeprazole treatment, except that the effect of amoxicillin and omeprazole was studied only in those subjects who were HP seropositive at baseline. *P* values for comparisons of mean concentrations of the 3 analytes were obtained by using the unpaired *t* test.

The principal analysis of the main effects of supplementation with garlic or vitamin and selenium was based on a linear regression with these main effects and with main effects for sex, age, age^2, body mass index (BMI; in kg/m^2), BMI^2, and 2 indicators for the strata: HP seropositivity at baseline with amoxicillin and omeprazole treatment and HP seronegativity at baseline with the placebo for amoxicillin and omeprazole. The subjects who were HP seropositive at baseline and who received the placebo for amoxicillin and omeprazole formed the reference stratum. The main analysis for amoxicillin and omeprazole effects was confined to subjects who were HP seropositive at baseline and was adjusted for sex, age, age^2, BMI, BMI^2, and main effects for garlic and vitamin and selenium supplements. Only main-effects models were presented because no significant interactions were found. The models were fitted separately for the serum lipid measurements in 1999 and 2003 on subjects with lipid measurements in both years. Because the persons contributing to these analyses had the same covariates and design matrix *X*, with projection operator

$$L = (X'X)^{-1}X' \tag{1}$$

then the difference in parameter estimates is

$$\hat{\beta}_1 - \hat{\beta}_2 = L(Y_1 - Y_2) \tag{2}$$

with covariance:

$$\Omega = LL'\hat{V}ar(Y_{1i} - Y_{2i}) \tag{3}$$

Here indexes 1 and 2 correspond to 1999 and 2003, respectively, and $\hat{V}ar(Y_{1i} - Y_{2i})$ is the sample variance of the differences $(Y_{1i} - Y_{2i})$ over persons indexed by *i*. The appropriate element of Ω is a variance that can be used to test whether an intervention had the same effect in both 1999 and 2003. Regression analyses were repeated by including, in addition to the factors above, the amounts of dietary garlic intake and dietary vegetable (ie, Chinese cabbage, potatoes, turnips, tomatoes, and apples) intake at baseline in 1995. The magnitude of the intervention effect was re-expressed as a percentage of the mean 1999 analyte concentrations. To identify the largest plausible beneficial effect of treatment, the lower 95% confidence limit was also divided by the mean 1999 analyte concentration.

Secondary analyses of all-cause and CVD mortality were done by using Cox proportional hazards regression to estimate relative risks. This intention-to-treat analysis was based on all of the 3411 randomly assigned subjects except 44 who were subsequently found to have had cancer before randomization and 2 who had died before randomization. The first type of exclusion should have rendered these subjects ineligible for randomization, according to the protocol, and the second type also did so. CVD

deaths included deaths due to coronary heart disease, hypertensive heart disease, rheumatic heart disease, pulmonary heart disease, and cerebrovascular diseases. Follow-up ended on 1 May 2003.

RESULTS

The treatment allocations in the $2 \times 2 \times 2$ and the 2×2 factorial strata of the SIT were balanced on age and sex (6). In the current study population, the mean $\pm$ SEM age in 1999 was 51.27 ± 0.61 and 51.37 ± 0.60 y in the active and placebo garlic groups, 50.62 ± 0.60 and 52.02 ± 0.61 y in the active and placebo vitamin and selenium groups, and 51.05 ± 0.76 and 50.35 ± 0.72 y in the active and placebo amoxicillin and omeprazole groups, respectively. The mean BMI measures were 23.20 ± 0.22 and 23.34 ± 0.23 in the active and placebo garlic groups, 23.28 ± 0.23 and 23.25 ± 0.22 in the active and placebo vitamin and selenium groups, and 23.35 ± 0.27 and 23.15 ± 0.28 in the active and placebo amoxicillin and omeprazole groups, respectively. The mean proportions of females were $41.7 \pm 3.5\%$ and $50.0 \pm 3.5\%$ in the active and placebo garlic groups, $48.0 \pm 3.5\%$ and $43.6 \pm 3.5\%$ in the active and placebo vitamin and selenium groups, and $52.9 \pm 4.3\%$ and $44.1 \pm 4.3\%$ in the active and placebo amoxicillin and omepraxole groups, respectively. The differences between the active and placebo groups were not significant with respect to these and other baseline variables (data not shown).

Approximately 95% of subjects took all their pills, and serum concentrations of *S*-allylcysteine, vitamin C, and α-tocopherol were higher in the active arms than in placebo controls in quarterly samples from 80 randomly selected subjects (8, 9). Of the 3365 randomly assigned, eligible SIT subjects included in the analysis of survival data, 183 died during follow-up, including 51 who died of CVD; 34 were lost to follow-up.

Effects of supplementation

Garlic

Descriptive analyses found that, in 1999, after 3.3 y of garlic supplementation, the difference in means (garlic minus placebo) was -0.11 (95% CI: -0.32, 0.10), -0.02 (95% CI: -0.09, 0.06), and -0.06 (95% CI: -0.24, 0.11) for total, HDL, and LDL cholesterol, respectively (**Table 2**). In 2003, after 7.3 y of supplementation, the corresponding values were -0.04 (95% CI: -0.22, 0.13), -0.02 (95% CI: -0.08, 0.05), and -0.04 (95% CI: -0.20, 0.12), respectively (Table 2). Regression analysis after adjustment for sex, age, age^2, BMI, BMI^2, vitamins, HP seropositivity (yes or no), and amoxicillin and omeprazole treatment strata yielded similar results; no significant differences in the mean concentrations of the 3 analytes were found between the active garlic and the garlic placebo groups in 1999 and 2003 (**Table 3**). Further adjustment for dietary garlic and vegetable intakes did not alter the results appreciably (data not shown). No significant differences between the active garlic and the garlic placebo groups were observed with respect to either all-cause or CVD mortality (**Table 4**).

Vitamins and selenium

After 3.3 y of vitamin and selenium supplementation, the difference in means (vitamin and selenium minus placebo) was 0.04 (95% CI: -0.17, 0.25), -0.05 (95% CI: -0.12, 0.03), and

TABLE 2
Total, HDL-, and LDL-cholesterol concentrations by treatment in 1999 and 2003

		1999			2003	
	n	$\bar{x} \pm$ SEM concentration	P^1	n	$\bar{x} \pm$ SEM concentration	P^1
Cholesterol (mmol/L)						
Garlic						
Yes	204	4.95 ± 0.07		190	4.84 ± 0.06	
No	204	5.06 ± 0.08		191	4.89 ± 0.07	
Difference in means		-0.11 ± 0.11	0.30		-0.04 ± 0.09	0.63
Vitamin and selenium						
Yes	204	5.02 ± 0.07		190	4.96 ± 0.06	
No	204	4.98 ± 0.08		191	4.77 ± 0.07	
Difference in means		0.04 ± 0.11	0.70		0.20 ± 0.09	0.03
Amoxicillin and omeprazole						
Yes	136	5.08 ± 0.11		131	4.91 ± 0.08	
No	136	4.93 ± 0.07		125	4.89 ± 0.07	
Difference in means		0.14 ± 0.13	0.28		0.02 ± 0.11	0.86
HDL (mmol/L)						
Garlic						
Yes	204	1.43 ± 0.03		190	1.53 ± 0.03	
No	204	1.44 ± 0.03		191	1.55 ± 0.02	
Difference in means		-0.02 ± 0.04	0.67		-0.02 ± 0.03	0.60
Vitamin and selenium						
Yes	204	1.41 ± 0.03		190	1.56 ± 0.02	
No	204	1.46 ± 0.03		191	1.52 ± 0.02	
Difference in means		-0.05 ± 0.04	0.20		0.03 ± 0.03	0.33
Amoxicillin and omeprazole						
Yes	136	1.47 ± 0.04		131	1.54 ± 0.03	
No	136	1.43 ± 0.03		125	1.56 ± 0.03	
Difference in means		0.04 ± 0.05	0.36		-0.02 ± 0.04	0.70
LDL (mmol/L)						
Garlic						
Yes	204	2.92 ± 0.06		190	2.92 ± 0.05	
No	204	2.98 ± 0.07		191	2.96 ± 0.06	
Difference in means		-0.06 ± 0.09	0.46		-0.04 ± 0.08	0.64
Vitamin and selenium						
Yes	204	2.97 ± 0.05		190	3.03 ± 0.06	
No	204	2.94 ± 0.07		191	2.86 ± 0.06	
Difference in means		0.03 ± 0.09	0.72		0.17 ± 0.08	0.04
Amoxicillin and omeprazole						
Yes	136	2.99 ± 0.08		131	2.98 ± 0.08	
No	136	2.88 ± 0.06		125	2.93 ± 0.06	
Difference in means		0.11 ± 0.10	0.28		0.05 ± 0.10	0.65

[1] Assessed by *t* test.

0.03 (95% CI: −0.12, 0.03) for total, HDL, and LDL cholesterol, respectively (Table 2). The corresponding values, after 7.3 y of supplementation, were 0.20 (95% CI: 0.02, 0.37), 0.03 (95% CI: −0.03, 0.10), and 0.17 (95% CI: 0.01, 0.33), respectively (Table 2). These differences were significant for total ($P = 0.03$) and LDL ($P = 0.04$) cholesterol after 7.3 y of supplementation (Table 2). Regression analysis after adjustment for sex, age, age^2, BMI, BMI^2, garlic supplementation, and amoxicillin and omeprazole treatment strata yielded similar results. The adjusted estimates of the effects of vitamin and selenium supplementation at 7.3 y were 0.22 mmol/L ($P = 0.01$) and 0.19 mmol/L ($P = 0.02$) for total and LDL-cholesterol concentrations, respectively (Table 3). Additional adjustments for dietary garlic and vegetable intakes yielded similar results (data not shown).

All-cause mortality did not differ significantly between SIT participants receiving vitamin and selenium supplementation and those receiving placebo (Table 4). A significantly lower CVD mortality rate (RR = 0.55, $P = 0.04$) was observed in subjects receiving vitamins and selenium (18 deaths) than in subjects receiving the placebo for vitamins and selenium (33 deaths) (Table 4).

Effect of amoxicillin and omeprazole treatment

Descriptive analysis of the data on the 1999 samples from subjects who were HP seropositive at baseline found that the mean differences (antibiotics minus placebo) for total, HDL, and LDL cholesterol were 0.14 (95% CI: −0.12, 0.40), 0.04 (95% CI: −0.05, 0.14), and 0.11 (95% CI: −0.09, 0.32), respectively (Table 2). Analyses of the 2003 samples yielded estimates of 0.02 (95% CI: −0.20, 0.24), −0.02 (95% CI: −0.10, 0.06), and 0.05 (95% CI: −0.15, 0.24), respectively (Table 2). Adjustment for

TABLE 3
Adjusted main treatment effects on total, HDL, and LDL cholesterol

	1999		2003		Equality of effects in 1999 and 2003
	Main effect	P	Main effect	P	P
	mmol/L		*mmol/L*		
Total cholesterol					
Garlic[1]	-0.11 ± 0.11[2]	0.32	-0.01 ± 0.09	0.91	0.29
Vitamin and selenium[1]	0.11 ± 0.11	0.29	0.22 ± 0.09	0.01	0.27
Amoxicillin and omeprazole[3]	0.12 ± 0.13	0.39	-0.03 ± 0.11	0.75	0.20
HDL					
Garlic[1]	-0.03 ± 0.04	0.36	-0.02 ± 0.03	0.46	0.76
Vitamin and selenium[1]	-0.04 ± 0.04	0.28	0.03 ± 0.03	0.32	0.03
Amoxicillin and omeprazole[3]	0.06 ± 0.05	0.23	-0.01 ± 0.04	0.76	0.12
LDL					
Garlic[1]	-0.06 ± 0.09	0.52	-0.003 ± 0.08	0.97	0.46
Vitamin and selenium[1]	0.10 ± 0.09	0.28	0.19 ± 0.08	0.02	0.19
Amoxicillin and omeprazole[3]	0.07 ± 0.10	0.47	-0.005 ± 0.09	0.96	0.37

[1] Analysis included 380 subjects with complete data on the analytes in 1999 and 2003 and on the covariates used for adjustment, ie, sex, age, age[2], BMI, BMI[2], *Helicobater pylori* seropositivity with amoxicillin and omeprazole treatment, and *H. pylori* seronegativity with placebo. Because no significant interactions were found, only main effects are presented.

[2] $\bar{x} \pm$ SEM (all such values).

[3] Analysis was confined to 255 subjects who were seropositive for *H. pylori* at baseline and had complete data on analytes in 1999 and 2003 and on covariates; the analysis was adjusted for sex, age, age[2], BMI, BMI[2], and the main effects of garlic or vitamin and selenium treatments. Because no significant interactions were found, only main effects are presented.

age, sex, BMI, and garlic or vitamin and selenium supplementation (or both) yielded similarly negative results (Table 3). Further analyses after adjustment for dietary garlic and vegetable intakes did not alter the results appreciably (data not shown). The amoxicillin and omeprazole treatment had no significant effects on all-cause or CVD mortality in the 2107 SIT participants who were HP seropositive at baseline (Table 4).

DISCUSSION

The current study is the first large-scale, placebo-controlled, randomized trial of long-term supplementation with garlic extract and garlic oil in a general population. The subjects in the current study were randomly selected from the general population of Linqu County, Shandong Province, China. In this rural Chinese population, we did not find a significant effect of garlic supplementation on serum concentrations of total, HDL, or LDL cholesterol after 3.3 and 7.3 y of intervention. From regression analyses, we estimated the respective intervention effects, re-expressed as percentages of the mean 1999 total, HDL-, and LDL-cholesterol concentrations, to be -2.4%, -2.1%, and -2.0%, respectively, after 3.3 y and -0.28%, -1.4%, and -0.30%, respectively, after 7.3 y of garlic supplementation. None of the intervention effects were statistically significant. Furthermore, the differences between the main effects observed in 1999 and those observed in 2003 were not significant for any of the interventions except vitamin and selenium supplementation, which increased HDL concentrations slightly in 2003 and lowered them slightly in 1999 (Table 3).

The trial was large enough ($n = 204$ receiving supplementation and 204 receiving placebo) to provide reasonably precise

TABLE 4
Proportional hazards analysis of time to all-cause and cardiovascular disease (CVD) mortality by treatment

Treatment	Subjects	All-cause mortality			CVD mortality[1]		
		Deaths	Hazard ratio[2] (95% CI)	P	Deaths	Hazard ratio[2] (95% CI)	P
	n	*n*			*n*		
Garlic							
Yes	1678	95	1.09 (0.82, 1.46)	0.56	27	1.14 (0.66, 1.97)	0.65
No	1687	88			24		
Vitamin							
Yes	1677	82	0.81 (0.61, 1.09)	0.17	18	0.55 (0.31, 0.97)	0.04
No	1688	101			33		
Amoxicillin and omeprazole							
Yes	1130	67	1.15 (0.81, 1.63)	0.44	18	1.19 (0.60, 2.36)	0.62
No	1128	58			15		

[1] Included deaths due to coronary heart disease, hypertensive heart disease, rheumatic heart disease, pulmonary heart disease, and cerebrovascular disease.
[2] Calculated by using Cox proportional hazards regression.

estimates. On the basis of the lower limits of the 95% CIs for the adjusted effects of garlic (Table 3), the largest plausible decreases consistent with our data for total, HDL, and LDL cholesterol were 6.7%, 7.5%, and 8.1%, respectively, at 3.3 y and 3.8%, 6.0%, and 5.4%, respectively, at 7.3 y.

Four meta-analyses of randomized, placebo-controlled human studies on hypocholesterolemic effects of garlic have been reported (4, 11–13). The most recent and most comprehensive of these studies (4) analyzed 13 randomized, placebo-controlled clinical trials that used a single garlic preparation, either garlic powder (11 studies), essential oil (1 study), or steam-distilled oil (1 study), and it required trial participants to have total cholesterol concentrations of ≥5.17 mmol/L (200 mg/dL). Ten of the 13 trials included patients with a primary diagnosis of hyperlipidemia; of the other 3 trials, 1 included patients with coronary heart disease, 1 included patients with hypertension, and 1 had healthy subjects. Study durations ranged from 8 to 24 wk, and, in most cases, total sample sizes (including placebo group) ranged from 25 to 62 participants, although 2 of the 13 trials had 115 and 221 subjects. The weighted mean difference in total cholesterol concentrations compared with placebo was −0.41 mmol/L (95% CI: −0.66, −0.15, $P < 0.01$). Although the weight of evidence in hypercholesterolemic populations suggested that garlic preparations lowered the total cholesterol concentrations by a small amount, this finding was not consistent across studies. For example, the cholesterol concentrations did not differ significantly between garlic and placebo groups as reported by the 6 diet-controlled studies that controlled for important confounding factors by monitoring patients' dietary intake and assessing compliance with the treatment regimen (4); the weighted mean difference was −0.11 (95% CI: −0.30, 0.08). In addition, 4 of the 5 clinical trials, which were excluded because of insufficient data for pooling, did not find a garlic effect. Furthermore, a recent 12-wk randomized, double-blind, placebo-controlled study of 75 normolipidemic men and women aged 40–60 y did not find an effect of garlic powder on total, LDL, or HDL cholesterol (5).

The subjects in the current study had lower meat intakes and slightly lower mean cholesterol concentrations than does the US population. They consumed ≈14 kg meat/y, whereas the average annual meat consumption in the United States is 61 kg. Study participants had mean ± SD 1999 concentrations of 5.00 ± 1.08, 1.43 ± 0.39, and 2.95 ± 0.88 mmol/L for total, HDL, and LDL cholesterol, respectively. These values were close to those reported for the Chinese population: 5.11 ± 0.87, 1.46 ± 0.39, and 3.01 ± 0.82 mmol/L, respectively (14). Corresponding values in the US population are 5.26, 1.32, and 3.19 mmol/L, respectively (15). Thus, the SIT population had only modestly lower concentrations of total and LDL cholesterol and modestly higher concentrations of HDL than are found in the United States. Although we used a different garlic preparation than did Spigelski and Jones (16), the data from the current study are consistent with their findings; they concluded from a literature review that consumption of garlic powder did not play a significant role in lowering plasma lipid concentrations in subjects following a low-fat, low-cholesterol diet. As indicated by the 95% CIs in Table 4, the current study had only modest power to detect effects of garlic supplementation on all-cause or CVD mortality, and no significant effects were found.

In the current study, long-term vitamin and selenium supplementation induced a small but significant increase in concentrations of total and LDL cholesterol. Mean HDL concentrations were not affected significantly; slight decreases and slight increases were found after 3.3 and 7.3 y, respectively. From regression analyses, the estimated respective percentage changes for total, HDL, and LDL cholesterol were 2.0%, −2.8%, and 2.7%, respectively, after 3.3 y and 4.2%, 2.1%, and 6.1%, respectively, after 7.3 y. On the basis of the lower limits of the CIs for the adjusted vitamin and selenium effects (Table 3), the smallest plausible percentage increases in total and LDL cholesterol were 0.8% and 0.9%, respectively, at 7.3 y. It is reassuring that no indication was found of greater risk of all-cause or CVD mortality in the vitamin and selenium supplementation group than in the placebo group, and, indeed, the evidence for CVD mortality is favorable (Table 4) despite the small increases in total and LDL cholesterol concentrations. Because we examined 3 treatments and 2 survival endpoints, and because the study had modest power to detect effects on mortality, the CVD finding is only suggestive.

A recent, 7.5-y, randomized placebo-controlled study of low-dose antioxidant supplementation (120 mg vitamin C, 30 mg vitamin E, 6 mg β-carotene, 100 μg selenium, and 20 mg zinc) in middle-aged adults free of CVD and cancer at baseline found no significant difference in the effect on mean cholesterol between treated and placebo groups (17). Another uncontrolled study of 39 healthy volunteers who received 600 IU vitamin E for 30 d reported a significant ($P = 0.01$) increase (≈5%) in total cholesterol concentrations (18). In 2 observational studies, vitamin C intake in healthy adults was associated with high HDL (19) and low cholesterol (20) concentrations. Another observational study did not find such associations (21). We found no data on the effect of selenium on serum lipids.

Vitamin E is believed to be a powerful antioxidant that protects cells against free radicals, and several CVD studies suggested protective effects of vitamin E (22). Recent data from the Women's Health Study, a randomized controlled trial among 39 876 low-risk women treated with 600 IU vitamin E on alternate days, indicated a 24% reduction (RR = 0.76; 95% CI: 0.59, 0.98; $P = 0.03$) in CVD deaths, but no reduction in major CVD events (23). In contrast, a recent meta-analysis of controlled clinical trials of vitamin E supplementation in high-risk patients reported significantly greater mortality in those receiving >400 IU of vitamin E (24), which is twice the daily dose of 200 IU used in SIT, than in those receiving placebo. Moreover, several recent randomized controlled trials, 2 of which were in apparently healthy populations (25, 26), reported that vitamin E did not help to prevent CVD events. The data in Table 4, based on only 51 CVD deaths, do not add significant information on the effects of vitamin E compared with larger studies, but the trend, which could also be related to vitamin C or selenium, is favorable.

We found no effects, 3.5 and 7.5 y later, of one-time treatment with amoxicillin and omeprazole on serum lipids. These results are not unexpected and are consistent with the findings of a 3-mo prospective study of 686 HP-positive patients who received one-time triple therapy for HP infection (a 7-d course of 20 mg omeprazole twice daily; 500 mg clarithromycin twice daily; and 1 g amoxicillin twice daily). That study reported no noticeable influence on serum lipids after control for changes in lifestyle (27).

In conclusion, in our randomized, controlled trial in a general adult population in rural China, neither long-term garlic supplementation nor short-term antibiotic treatment had an appreciable effect on serum cholesterol or lipoprotein concentrations or on all-cause or CVD mortality. Serum total and LDL-cholesterol

concentrations were modestly but significantly higher after long-term supplementation with vitamins E and C and selenium than after receiving placebo, but no associated increase in all-cause or CVD mortality was found. Thus, our data do not support the use of this garlic preparation or of vitamin E or C or selenium supplementation to lower serum concentrations of total or LDL cholesterol or to increase concentrations of HDL cholesterol in persons in this rural Chinese population. Although the meat intake in this population was substantially lower than that in the United States, the mean total cholesterol concentration was only 5% lower. Thus, these findings may provide better guidance for the general US population than do studies of subjects with CVD or elevated cholesterol.

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