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Long-Term Garlic or Micronutrient Supplementation, but Not Anti-*Helicobacter pylori* Therapy, Increases Serum Folate or Glutathione Without Affecting Serum Vitamin B-12 or Homocysteine in a Rural Chinese Population[1–4]

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Abstract

The effects of a 7.3-y supplementation with garlic and micronutrients and of anti-*Helicobacter pylori* treatment with amoxicillin (1 g twice daily) and omeprazole (20 mg twice daily) on serum folate, vitamin B-12, homocysteine, and glutathione concentrations were assessed in a rural Chinese population. A randomized, double-blind, placebo-controlled, factorial trial was conducted to compare the ability of 3 treatments to retard the development of precancerous gastric lesions in 3411 subjects. The treatments were: *1*) anti-*H. pylori* treatment with amoxicillin and omeprazole; *2*) 7.3-y supplementation with aged garlic and steam-distilled garlic oil; and *3*) 7.3-y supplementation with vitamin C, vitamin E, and selenium. All 3 treatments were given in a 2^3 factorial design to subjects seropositive for *H. pylori* infection; only the garlic supplement and vitamin and selenium supplement were given in a 2^2 factorial design to the other subjects. Thirty-four subjects were randomly selected from each of the 12 treatment strata. Sera were analyzed after 7.3 y to measure effects on folate, vitamin B-12, homocysteine, and glutathione concentrations. Regression analyses adjusted for age, gender, and smoking indicated an increase of 10.2% (95%CI: 2.9–18.1%) in serum folate after garlic supplementation and an increase of 13.4% (95%CI: 5.3–22.2%) in serum glutathione after vitamin and selenium supplementation. The vitamin and selenium supplement did not affect other analytes and the amoxicillin and omeprazole therapy did not affect any of the variables tested. In this rural Chinese population, 7.3 y of garlic supplementation increased the serum folate concentration and the vitamin and selenium supplement increased that of glutathione, but neither affected serum concentrations of vitamin B-12 or homocysteine. J. Nutr. 139: 106–112, 2009.

Introduction

Increased serum concentrations of homocysteine have been associated with an increased risk of cardiovascular disease (1), other vascular conditions (2), and cancer (3). Folate and vitamin B-12 are essential nutrients whose deficiency causes neurological disorders (4) and has been associated with cardiovascular and cerebrovascular diseases (5) as well as cancer (6). Glutathione is involved in the detoxification of exogenous and endogenous carcinogens, free radicals, and other sources of oxidative stress and its deficiency has been associated with cardiovascular disease (7), arthritis (8), and malignancies (9). This analyte is also involved in maintaining the immune system (10–12).

Pathways involving folate, vitamin B-12, homocysteine, and glutathione are complex (13–17). These 4 micronutrients par-

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[4] Supplemental Table 1 is available with the online posting of this paper at jn.nutrition.org.
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ticipate jointly in several metabolic cycles, including the 1-carbon cycle for synthesis and methylation of DNA and other biologic compounds (18). Folate is one of the main coenzymes and a 1-carbon carrier/donor for some of the reactions, along with vitamin B-12. Homocysteine and glutathione are 2 of the main nutritional substrates in this cycle and their levels are maintained by various homeostatic reactions. In particular, glutathione levels are regulated by reactions of the pentose phosphate pathway (19).

In humans, the effects of long-term garlic supplementation on the serum concentrations of folate, vitamin B-12, homocysteine, and glutathione are not known. Likewise, little is known about the long-term effects of supplementation with vitamin E, C, and selenium on these micronutrients, although short-term effects on glutathione have been noted (see "Discussion"). Finally, the long-term effects of amoxicillin and omeprazole antibiotic therapy for *Helicobacter pylori* on these 4 micronutrients are not known. However, omeprazole, a proton pump inhibitor, has been associated with decreased vitamin B-12 concentrations in several studies (20) and *H. pylori* eradication has been associated with increased serum folate and vitamin B-12 concentrations and decreased homocysteine concentrations (21–23). A study of *H. pylori* infection in patients undergoing diagnostic coronary arteriography suggested that *H. pylori*-induced chronic atrophic gastritis (CAG)[11] decreases plasma vitamin B-12 and folic acid concentrations, thereby increasing homocysteine concentrations (21). Because of the possible etiologic role of these micronutrients in several human diseases, there is considerable interest in determining whether dietary factors such as garlic or vitamin supplements influence their serum concentrations.

In 1995, the National Cancer Institute and the Beijing Institute for Cancer research 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 (GC). Those treatments were amoxicillin and omeprazole (only in subjects with *H. pylori* infection), dietary supplementation with vitamins E and C and selenium, and dietary supplementation with steam-distilled garlic oil and ethanol-aqueous aged garlic extract (AGE). These interventions were chosen partly on the basis of previous studies in Shandong that showed decreased risk of GC with increased consumption of garlic and other allium-containing vegetables (24) and with increased consumption of fruit and vegetables (25), as well as decreased risk of advanced precancerous gastric lesions with higher serum concentrations of vitamin C (26). We have previously published studies on the design of the SIT (27) and on treatment effects on precancerous gastric lesions (28) and serum lipoproteins (29). The study population of 3411 subjects in the SIT was representative of the general population in Linqu County, Shandong Province, China (27,28).

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 for 7.3 y had any effect on serum concentrations of folate, vitamin B-12, homocysteine, or glutathione 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. A secondary objective was to examine the effect of anti-*H. pylori* therapy with amoxicillin and omeprazole on these serum micronutrient concentrations. This double-blind, randomized study permitted the assessment of associations of supplement intake with serum micronutrient concentrations while avoiding selection biases that may confound observational studies.

[11] Abbreviations used: AGE, aged garlic extract; CAG, chronic atrophic gastritis; GC gastric cancer; SIT, Shandong Intervention Trial.

Methods

Study population and sampling design. The design of the SIT trial as well as the results of the effect of intervention on the occurrence of precancerous gastric lesions have been described elsewhere in detail (27). Briefly, a total of 3411 subjects were recruited into the trial and were randomized into 12 treatment strata, corresponding to a 2^3 factorial design in subjects who were seropositive for *H. pylori* at baseline in 1994 and a 2^2 factorial design for those who were seronegative for *H. pylori* at baseline (**Supplemental Table 1**).

For the study of serum nutrients, we subsampled the 3186 participants with sera from 1999 to obtain 408 subjects; 34 such subjects were randomly selected within each of the 12 treatment strata (Supplemental 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 (29). A total of 381 of the 408 subjects also had sera available from 2003 (Supplemental Table 1). The concentrations of folate, vitamin B-12, homocysteine, and glutathione were measured in these 381 serum samples. This study was approved by the institutional review boards of the Beijing Institute for Cancer Research and the U.S. National Cancer Institute and written informed consent to participate was obtained from each subject.

Treatments. Intervention with amoxicillin and omeprazole was given for 2 wk in 1995, whereas garlic (Kyolic, Wakanuga of America) and vitamin and selenium supplementation (Sino-American Shanghai Squibb Pharmaceuticals) were given for 88 mo from 1995 to 2003. The 3 interventions were: *1*) anti-*H. pylori* treatment with amoxicillin (1 g twice daily) and omeprazole (20 mg twice daily) for 2 wk; 6 wk after the initial 2-wk course, the subjects were given a ^{13}C-urea breath test and, if evidence of *H. pylori* infection persisted, a 2nd 2-wk course of treatment was offered; *2*) 2 capsules twice daily, each containing 200 mg ethanol-aqueous AGE and 1 mg steam-distilled garlic oil; and *3*) vitamin and selenium supplements containing 250 mg vitamin C, 100 mg vitamin E (α-tocopherol), and 37.5 μg selenium (yeast-selenium) twice daily. Placebos for each of these interventions were given when the treatment was not assigned. The doses of vitamins and selenium were chosen to exceed modestly the doses used in a previous intervention trial in Henan Province, China, that demonstrated a protective effect on GC mortality (30).

Compliance among the subjects was monitored by monthly counting of unconsumed pills left in the bottles and by quarterly measurements of serum concentrations of *S*-allyl cysteine, vitamin C, and α-tocopherol in sera from 80 randomly sampled subjects (31).

Measurement of serum folate, vitamin B-12, homocysteine, and glutathione. Five milliliters of blood were collected from each fasting SIT subject in 2003. The blood specimen was obtained in the morning and was allowed to clot in the dark at room temperature for 30–40 min and then centrifuged at $1000 \times g$ for 15 min. Serum samples were divided into aliquots in 3 vials that were stored immediately in a -20°C freezer. The serum samples were transferred in dry-ice to Beijing within 2 or 3 d after collection and kept in a -70°C freezer thereafter. Coded samples were sent to the laboratories so that the micronutrients were measured without knowledge of the characteristics or clinical status of the subjects. The serum concentrations of folate and vitamin B-12 were measured in February, 2005 at the Clinical Laboratory of Peking University People's Hospital and homocysteine and glutathione were measured in September, 2004 at the Beijing Institute for Cancer Research. Folate and vitamin B-12 were assayed using the electrochemiluminescence immunoassay with folate kit and vitamin B-12 kit on the E170 immunoassay analyzers (Roche Elecsys Modular Analytics).

An HPLC procedure (32) was used to measure serum total homocysteine and glutathione. The sample was measured on an HP-1050 HPLC system. A reversed-phase Zorbax Eclipse XDB-C8 (150 ×

4.6 mm, 5μm) column was placed in the thermostat at 30°C. A mobile phase of acentonitrile and potassium phosphate buffer (pH 3.78) was used in a programming sequence mode. The injection volume was 10 μL and peaks of homocysteine- and glutathione-thionitrobenzoate (which are derivatives of homocysteine and glutathione, respectively) were detected at 330 nm.

Quality control was performed for assays of folate, vitamin B-12, homocysteine, and glutathione as follows. For each sample measurement, a duplicate test was performed. If the difference between the duplicate values exceeded 5%, a repeat assay was conducted to confirm the results. Standard samples with high and low levels of the nutrients were analyzed daily to ensure that the assay procedures were within quality control limits and to calibrate the equipment. The CV of folate, vitamin B-12, homocysteine, and glutathione assays were 4.5, 8.4, 12.5, and 7.7%, respectively.

Statistical analysis. Analyses were performed on an intention-to-treat basis (i.e. all randomized subjects were analyzed in the treatment group to which they were assigned at randomization regardless of the subject's degree of compliance). Two-sided, 0.05-level *P*-values were used for all analyses. Analyses were performed using SAS (release 9.0, SAS Institute) statistical software.

Medians with interquartile ranges of serum concentrations of folate, vitamin B-12, homocysteine, and glutathione were calculated in those assigned to the garlic supplement and garlic placebo groups and differences in the analyte distributions were assessed with a 2-sided Wilcoxon's rank sum test. A similar comparison was performed for vitamin and selenium supplementation and for amoxicillin and omeprazole treatment, except that the amoxicillin and omeprazole effect was studied in only those who were initially *H. pylori* seropositive.

The principal analysis for the main intervention effects used robust regression [SAS Proc Robustreg, option MM (33)]. The dependent variable was log10 (analyte), because the distributions of log-transformed values were more nearly normally distributed than the original values. The model for garlic included an indicator variable for garlic supplements and was analyzed on the 378 subjects with complete analyte data in 2003. A similar model was used with an indicator for vitamin and selenium supplements on these subjects. A model with an indicator for amoxicillin and omeprazole treatment was applied to those 256 subjects who were initially *H. pylori* seropositive and who had complete analyte data. Each of these 3 models also included age in 1994, gender, and smoking (ever vs. never) as main effects. The treatment effect in these models was the coefficient of the treatment indicator and is an adjusted estimate of the logarithm to the base 10 of the ratio of the analyte concentration on treatment to the analyte concentration on placebo. To study the joint effects of the interventions, we additionally included all main effects and 2-way interactions among the interventions for the analysis of amoxicillin and omeprazole treatment. For the analyses of garlic and vitamin interventions, we included interactions among these 2 interventions and with the 2 strata indicators defined for subjects who were initially *H. pylori* seropositive and receiving amoxicillin and omeprazole treatment and for subjects who were initially *H. pylori* seronegative. An estimated treatment effect on the log10 scale, Δ, was converted into a percentage increase (or decrease) in the treatment group compared with placebo via the formula 100(10Δ −1). CI on the percentage increase (or decrease) were calculated by substituting the lower and upper 95% confidence limits for Δ in this formula.

Results

Treatment balance on covariates and treatment compliance. Age and gender distribution did not differ in all treatment vs. placebo comparisons (**Table 1**), as anticipated from the stratified design (27). The randomization also led to good treatment balance on smoking status and baseline histopathology, variables that did not differ between the treatment and placebo groups (29).

About 95% of subjects took all their pills, and serum concentrations of *S*-allyl cysteine, vitamin C, and α-tocopherol were higher in the treatment groups than in placebo controls in quarterly samples from 80 randomly selected subjects (31).

Population concentrations of micronutrients. Median serum concentrations of folate were 14.8 nmol/L in the garlic placebo group and 15.0 nmol/L in the vitamin placebo group (**Table 2**). The proportions with serum folate concentrations <13.6 nmol/L, considered to be an indication of mild folate deficiency (34), were 39.8 and 36.1%, respectively, for the garlic and vitamin placebo groups. Thus, there was evidence of mild folate deficiency in this population. The corresponding proportions with homocysteine concentrations >15 μmol/L, an indication of folate deficiency (34), were 14.7 and 20.9%. Median concentrations of glutathione were 1.91 and 1.80 μmol/L in the garlic and vitamin placebo groups, respectively (Table 2). The proportions with vitamin B-12 concentrations <185 pmol/L, an indication of deficiency (34), were 41.9 and 43.5% in the garlic and vitamin placebo groups, respectively.

Effects of garlic supplementation. The median serum folate concentration was lower in the placebo group than in the group assigned to garlic supplements ($P = 0.017$) (Table 2). There were no other significant effects of garlic supplementation in these unadjusted analyses. Regression analyses adjusted for age, gender, and smoking confirmed these findings (**Table 3**) and indicated that garlic supplementation increased folate by 10.2% (95%CI: 2.9–18.1%) ($P < 0.006$).

Effects of vitamins and selenium supplementation. The median serum glutathione concentrations in those assigned to the vitamin and selenium supplementation group was greater than the concentration in the placebo group ($P < 0.001$) (Table 2). None of the other analytes were affected by the interventions in these unadjusted analyses, but the serum homocysteine concentration tended to be lower in the treated group than in the placebo group ($P = 0.06$). These patterns were also seen after adjustment for age, gender, and smoking (Table 3). Serum glutathione was increased by 13.4% (95%CI: 5.3–22.2%) ($P < 0.001$) and the plasma homocysteine concentration tended to be decreased −6.4% (95%CI: −13.5 to 1.3%) ($P = 0.10$).

Effect of amoxicillin and omeprazole treatment. In the entire SIT population treated with amoxicillin and omeprazole, the eradication rate, based on the initial 2-wk treatment with one 2-wk retreatment of those who failed initially, was 827/1130 (73%) (28) and 7 y later in 2003, 47% of the group treated with amoxicillin and omeprazole remained negative on the ^{13}C-urea breath test (35). For the subset of those SIT subjects included in the current study, the initial eradication rate was 94/131 (72%) and 43% remained negative in 2003.

Short-term treatment with amoxicillin and omeprazole in 1995 reduced the serum concentration of vitamin B-12 in 2003 ($P = 0.027$) in unadjusted analyses (Table 2). However, this was not significant after adjustment for age, gender, and smoking status (Table 3). The adjusted estimate of percentage change in vitamin B-12 compared with placebo was −10.2% (95%CI: −21.7 to 2.9%) ($P = 0.12$). Amoxicillin and omeprazole treatment did not affect the other variables tested.

Joint effects of interventions. The factorial design assures good balance for each intervention across all other interventions (Supplemental Table 1). This balance protects against confounding by other interventions when estimating the main effect of each intervention (Tables 2 and 3). None of the interactions

TABLE 1 Baseline characteristics in 1994 of the sampled subjects with measurements in 2003[1]

	Garlic extracts		Vitamins C and E and selenium		Amoxicillin and omeprazole	
	Placebo	Treatment	Placebo	Treatment	Placebo	Treatment
n	191	190	191	190	125	131
Age, *y*	46.2 ± 8.5	46.2 ± 8.5	46.9 ± 8.6	45.5 ± 8.3	45.2 ± 8.1	46.1 ± 8.7
Gender				*n* (%)		
Male	95 (50)	111 (58)	106 (56)	100 (53)	70 (56)	61 (47)
Female	96 (50)	79 (42)	85 (44)	90 (47)	55 (44)	70 (53)
Smoking						
Never	111 (58)	89 (48)	101 (53)	99 (52)	66 (53)	72 (55)
Ever	80 (42)	98 (52)	88 (47)	90 (48)	59 (47)	59 (45)
Pathological diagnosis						
Normal	0	0	0	0	0	0
SG[2]	6 (3.2)	7 (3.7)	5 (2.6)	8 (4.3)	4 (3.2)	4 (3.1)
Mild or moderate CAG	86 (45.5)	85 (45.0)	79 (41.6)	92 (48.9)	45 (36.3)	45 (34.9)
Severe CAG	8 (4.2)	5 (2.6)	8 (4.2)	5 (2.7)	5 (4.0)	6 (4.6)
Superficial IM	13 (6.9)	18 (9.5)	16 (8.4)	15 (8.0)	13 (10.5)	11 (8.5)
Deep IM	53 (28.0)	53 (28.0)	55 (29.0)	51 (27.1)	43 (34.7)	45 (34.9)
Mild DYS	21 (11.1)	20 (10.5)	25 (13.2)	16 (8.5)	13 (10.5)	17 (13.2)
Moderate DYS	0 (0.0)	1 (0.5)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)
Severe DYS	1 (0.5)	0 (0.0)	1 (0.5)	0 (0.0)	1 (0.8)	0 (0.0)
GC	1 (0.5)	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	1 (0.8)

[1] Values are means ± SD or *n* (%).
[2] Abbreviations: SG, superficial gastritis; IM, intestinal metaplasia; DYS, dysplasia.

among treatments was significant, indicating that the effects of the interventions on the $\log_{10}$ (analyte) were additive and could be estimated by simply adding the effects in Table 3.

Discussion

This study is, to our knowledge, the first large-scale, placebo-controlled, randomized trial of long-term supplementation with garlic extract and garlic oil in a general population and the first human study on the effect of garlic supplementation on folate levels. We adjusted the analyses of serum folate, vitamin B-12, homocysteine, and glutathione for age, gender, and smoking; smoking has been shown to elevate homocysteine and reduce folate levels in human studies (36). In this rural Chinese population, supplementation with garlic extracts for 7.3 y raised the serum concentration of folate by 10.2% (95%CI: 2.9–18.1%), and vitamin and selenium supplementation for 7.3 y raised glutathione concentrations by 13.4% (95%CI: 5.3–22.2%).

Our findings for garlic supplementation are supported by animal studies. Yeh et al. (37) reported slight increases in plasma folate concentrations in rats with moderate folate deficiency following treatment with AGE. Treatment with AGE resulted in decreased plasma homocysteine concentrations among rats with severe folate deficiency (37,38) but not in rats with moderate folate deficiency. These results parallel our findings of increased folate concentrations but no significant decrease in homocysteine concentrations in the subjects given garlic supplementation who had only moderate folate deficiency. Our negative findings for homocysteine are in agreement with a recent randomized, double-blind, placebo-controlled clinical trial reporting no significant effect of garlic extract therapy on homocysteine in pediatric patients with familial hyperlipidemia (39) and with other negative reports (1).

TABLE 2 Serum concentrations of folate, glutathione, homocysteine, and vitamin B-12 in 2003 in rural Chinese who had received placebo or garlic, vitamin and selenium, or amoxicillin and omeprazole treatments[1]

	Garlic extracts			Vitamins C and E and selenium			Amoxicillin and omeprazole		
Analyte	Treatment	Placebo	*P*[2]	Treatment	Placebo	*P*[2]	Treatment	Placebo	*P*[2]
n	190	191		190	191		131	125	
Folate, *nmol/L*	16.3	14.8	0.017	15.8	15.0	0.73	15.8	16.4	0.67
	(13.2,19.8)	(12.3,19.9)		(13.0,19.9)	(12.6,19.7)		(12.8,21.0)	(13.2,21.0)	
Glutathione, *μmol/L*	1.96	1.91	0.89	2.09	1.80	<0.001	1.95	1.86	0.67
	(1.58,2.47)	(1.55,2.47)		(1.64,2.65)	(1.48,2.31)		(1.54,2.39)	(1.48,2.47)	
Homocysteine, *μmol/L*	10.2	9.56	0.26	9.35	10.3	0.06	9.45	9.90	0.55
	(7.59,14.4)	(7.69,12.3)		(7.39,12.3)	(8.01,14.5)		(7.24,13.2)	(7.79,12.3)	
Vitamin B-12, *pmol/L*	201	201	0.26	193	206	0.86	185	221	0.027
	(136,279)	(143,301)		(138,289)	(137,296)		(133,279)	(152,304)	

[1] Values are medians (interquartile range).
[2] Two-sided Wilcoxon's rank sum test *P*-value.

TABLE 3 Adjusted treatment effects for $\log_{10}$-transformed serum concentrations of folate, glutathione, homocysteine, and vitamin B-12 in rural Chinese who had received placebo or garlic, vitamin and selenium, or amoxicillin and omeprazole treatments and percent difference from placebo-treated controls[1]

Treatment	*n*	Serum analyte	Treatment effect (SEE)	*P*-value	Percent change (95% CI)
Garlic extracts	Treatment, 190 Placebo, 191	Folate	0.042 (0.015)	<0.006	10.2 (2.9 to 18.1)
		Glutathione	−0.007 (0.017)	0.67	−1.6 (−8.7 to 6.1)
		Homocysteine	0.015 (0.018)	0.40	3.4 (−4.6 to 12.2)
		Vitamin B-12	−0.016 (0.025)	0.51	−3.7 (−13.9 to 7.7)
Vitamins C and E and selenium	Treatment, 190 Placebo, 191	Folate	0.010 (0.016)	0.50	2.4 (−4.5 to 9.9)
		Glutathione	0.055 (0.016)	<0.001	13.4 (5.3 to 22.2)
		Homocysteine	−0.028 (0.018)	0.10	−6.4 (−13.5 to 1.3)
		Vitamin B-12	−0.009 (0.025)	0.71	−2.1 (−12.5 to 9.5)
Amoxicillin and omeprazole	Treatment, 131 Placebo, 125	Folate	−0.001 (0.020)	0.95	−0.3 (−9.0 to 9.2)
		Glutathione	0.003 (0.021)	0.88	0.0 (−8.2 to 10.5)
		Homocysteine	−0.013 (0.020)	0.51	3.0 (−11.6 to 6.4)
		Vitamin B-12	−0.047 (0.030)	0.12	−10.2 (−21.7 to 2.9)

[1] Robust regression [MM option of SAS Procedure Robustreg (32)] was used to estimate treatment effects adjusted for age, gender, and smoking (see "Methods"). The percent change, which is derived from the treatment effect as described in Methods, is 100× [(active treatment value/placebo treatment value) −1].

In the current study, long-term vitamin C and E and selenium supplementation induced a significant 13% increase in concentrations of glutathione. Several recent studies have investigated the effects of vitamins E and C and selenium on the levels of homocysteine, vitamin B-12, folate, and glutathione. A double-blind, randomized, placebo-controlled trial of selenium supplementation (247 μg/d) for 9 mo reported significantly increased levels of glutathione (40). A randomized trial of vitamin E supplementation for 4 wk also indicated significantly elevated levels of glutathione in 2 high-dose groups (60 and 200 mg/d) but not in the low-dose group (15 mg) (41). Another double-blind clinical trial reported significantly elevated levels of glutathione among diabetic children following vitamin E supplementation (100 mg/d) for 3 mo (42). High-dose (1000 mg/d), orally administered vitamin E was associated with increased levels of glutathione in human RBC in another study (43). Vitamin E and selenium together increased glutathione to normal levels in glutathione-deficient rats (44). Vitamin E and selenium could increase glutathione synthesis, because they are both components of glutathione synthase and glutathione peroxidase and may also regulate other enzymes affecting glutathione synthesis (41). Alternatively, they could reduce utilization of glutathione for the detoxification of free radicals, because both are antioxidants. Puskas et al. (45) reported that administration of dehydroascorbate, the major derivative of vitamin C in blood cells, increased glutathione levels and postulated that this increase resulted from stimulation of the pentose phosphate pathway. Thus, our finding of increased serum glutathione concentrations following vitamin and selenium supplementation is consistent with some previous reports and may be due to selenium, vitamin E, vitamin C, or to some combination of these micronutrients.

In the current study, the anti-*H. pylori* treatment with amoxicillin and omeprazole was not associated with changes in any analyte after adjustment for age, gender, and smoking (Table 3). However, serum concentrations of vitamin B-12 were lower in those subjects receiving treatment in unadjusted analyses (Table 2). Previous studies of the effect of omeprazole therapy on vitamin B-12 levels clearly show that omeprazole can lead to vitamin B-12 malabsorption by suppressing acid secretion and inhibiting the cleavage of vitamin B-12 from dietary proteins (20,46). On the other hand, a decline in gastric acid secretion and malabsorption is a cause of vitamin B-12 deficiency (47) and a reduction in precancerous gastric lesions in the group treated with amoxicillin and omeprazole (28) might lead to better absorption of vitamin B-12. Therefore, our findings of a nonsignificant decrease in serum vitamin B-12 (Table 3) may be due to chance or to a residual effect of omeprazole, although the latter seems unlikely after 7 y. The failure to demonstrate long-term effects of treatment with amoxicillin and omeprazole on folate and homocysteine (21–23) in our study is not due to the inability of this combination to eradicate *H. pylori*; this combination eradicated *H. pylori* in 72% of the treated patients in this study and 43% remained free of *H. pylori* at the end of the study in 2003 (28).

Our study has particular strengths and weaknesses. A major strength is the random allocation of interventions, which avoids the confounding from the self-selection that arises in observational studies of individuals who choose whether or not to take supplements. Treatment and control groups were well balanced with respect to age, gender, and baseline histopathology (27,28). Our analyses were adjusted for age, gender, and smoking, but this random allocation also reduced the potential for confounding by the many other measured and unmeasured factors that could influence these micronutrients, such as dietary intake, alcohol consumption, caffeine intake, and renal function. Unpublished data indicated stable dietary consumption patterns from 1995 to 2003 for fruits, vegetables, and allium-containing vegetables. Consumption of sweet and of sour pancakes decreased and meat consumption increased. All these trends were similar in treated and untreated groups. It is therefore likely that the randomization would also protect treatment comparisons in the presence of other secular dietary trends. A potential weakness is the reported CV in laboratory measurements of 12.5% for homocysteine, which is higher than for the other analytes. However, because person-to-person variability contributed more to the variability of estimated intervention effects than sources of laboratory error, the standard errors for the estimated effects of homocysteine were similar in magnitude to those of folate and glutathione and somewhat smaller than those of vitamin B-12 (Table 3). In fact, based on these estimates of variability, we calculated that our study had a power of 0.90 with a 2-sided 0.05-level test to detect an increase in homocysteine of ≥16% or a decrease of ≤14%. A survey of the population of Linqu County in 1989 (26) revealed mean serum concentrations of 17.8 μmol/L for vitamin C, 22.3 μmol/L for

α-tocopherol, and 0.38 μmol/L for selenium. These concentrations are considerably lower than in Western countries, which should be considered when drawing generalizations from this study.

In regions such as Linqu County, Shandong Province of China, where nearly 40% of the population had serum folate concentrations < 13.6 nmol/L, our findings on the effects of garlic supplements and vitamin supplements may have public health implications. Individuals in the US with mild folate or glutathione deficiency might also benefit from supplementation.

In summary, our randomized, controlled trial in a general population in rural China demonstrated that supplementation for 7.3 y with garlic extracts (aged aqueous-ethanol extract of garlic and steam-distilled garlic oil) increased serum folate and long-term supplementation with selenium and vitamins C and E increased the serum glutathione concentration.

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