

## Effects of Vitamin/Mineral Supplementation on the Prevalence of Histological Dysplasia and Early Cancer of the Esophagus and Stomach: Results from the Dysplasia Trial in Linxian, China<sup>1</sup>

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### Abstract

Linxian, China has some of the highest rates of esophageal/gastric cardia cancer in the world, and epidemiological evidence suggests that chronically low intake of micronutrients may contribute to these high cancer rates. To examine whether supplementation with multiple vitamins and minerals can affect the occurrence of esophageal/gastric cardia cancer in this population, a two-arm randomized nutrition intervention trial was conducted among 3318 Linxian residents with cytological evidence of linxian dysplasia. During the 6-year intervention, esophageal/gastric cardia cancer mortality was 8% lower among those receiving the active supplements. After 30 and 72 months of intervention, endoscopic surveys were carried out to see if the nutritional supplements had affected the prevalence of clinically silent precancerous lesions and early invasive cancers of the esophagus and stomach. In the first survey, in 1987, 833 subjects were endoscoped; in the second survey, in 1991, 396 subjects were examined. The histological diagnoses from each survey were compared by treatment group. Cancer or dysplasia was diagnosed in 28% of those subjects endoscoped in 1987 and 24% of those examined in 1991. The odds ratio for subjects in the treatment group (*versus* those in the placebo group) having esophageal or gastric dysplasia or cancer was 0.84 (95% confidence interval, 0.61-1.15) in 1987 and 0.86 (0.54-1.38) in 1991. Although modest protective effects on worst overall diagnosis were seen in the supplemented group in both surveys, none of the results was statistically significant, and the findings must be considered inconclusive. It is likely that longer interventions and larger numbers of

endoscoped subjects will be required to fully investigate the effects of micronutrient supplementation in this population.

### Introduction

Previous reports have described a randomized multiple vitamin/mineral intervention trial among persons with cytologically diagnosed esophageal dysplasia in Linxian, a rural county in northern China which has some of the highest rates of esophageal/gastric cardia cancer in the world (1, 2). During this 6-year Dysplasia Trial, subjects receiving active supplements experienced nonsignificant reductions in total mortality (8%), esophageal cancer mortality (16%), and combined esophageal and gastric cardia cancer mortality (8%) and a nonsignificant increase in stomach cancer mortality (18%) when compared with subjects receiving placebo pills (2). In order to assess the effect of supplements on early asymptomatic cancers and premalignant lesions, endoscopic surveys were carried out in 1987 and 1991, after 30 and 72 months of intervention. This article reports the results of these two endoscopic surveys.

### Materials and Methods

#### Dysplasia Trial

Details of the procedures of the Dysplasia Trial have been previously described (1). In brief, 12,877 Linxian residents from Yaocun, Rencun, and Donggang communes were screened by esophageal balloon cytology in 1983 (3). The cytological categories in this screening were: Normal; Hyperplasia; Dysplasia 1 (low-grade); Dysplasia 2 (high-grade); Near Cancer; and Cancer. Of those screened, 3318 with a cytological diagnosis of dysplasia and no history of previous cancer, including 2545 (77%) with Dysplasia 1 and 773 (23%) with Dysplasia 2, were enrolled in the Dysplasia Trial and randomized into either the treatment or placebo group of this two-arm double-blind nutrition intervention trial. Active intervention, consisting of two high-potency multivitamin multimineral tablets (Centrum, Lederle Laboratories, Inc.) and one  $\beta$ -carotene capsule (15 mg as Solatene; Hoffmann-La Roche, Inc.) daily or matched placebos, began in May 1985 and ended in April 1991. The active pills contained 14 vitamins and 12 minerals, and daily doses, given in Table 1, were typically two to three times the U.S. Recommended Daily Allowances.

#### 1987 Endoscopic Survey

In November and December, 1987, after 30 months of active intervention, all Dysplasia Trial subjects with a 1983 cytology diagnosis of Dysplasia 2 and every fifth subject with a

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Table 1 Daily doses of micronutrients in the supplements of the Dysplasia Trial, Linxian, China<sup>a</sup>

Vitamin/mineral	Compound	Dose
$\beta$ -carotene		15 mg
Vitamin A	Acetate	10000 IU <sup>b</sup>
Vitamin E	<i>dl</i> -alpha tocopheryl acetate	60 IU
Vitamin C	Ascorbic acid	180 mg
Folic Acid		800 $\mu$ g
Vitamin B <sub>1</sub>	Thiamine mononitrate	5 mg
Vitamin B <sub>2</sub>	Riboflavin	5.2 mg
Niacinamide		40 mg
Vitamin B <sub>6</sub>	Pyridoxine HCL	6 mg
Vitamin B <sub>12</sub>	Cyanocobalamin	18 $\mu$ g
Vitamin D		800 IU
Biotin		90 $\mu$ g
Pantothenic acid	Calcium pantothenate	20 mg
Calcium	Dibasic calcium phosphate	324 mg
Phosphorus	Dibasic calcium phosphate	250 mg
Iodine	Potassium iodide	300 $\mu$ g
Iron	Ferrous fumarate	54 mg
Magnesium	Magnesium oxide	200 mg
Copper	Cupric oxide	6 mg
Manganese	Manganese sulfate	15 mg
Potassium	Potassium chloride	15.4 mg
Chloride	Potassium chloride	14 mg
Chromium	Chromium chloride	30 $\mu$ g
Molybdenum	Sodium molybdate	30 $\mu$ g
Selenium	Sodium selenate	50 $\mu$ g
Zinc	Zinc sulfate	45 mg

<sup>a</sup> Participants received 2 multivitamin, multimineral tablets (Centrum; Lederle Laboratories, Inc.) and 1  $\beta$ -carotene capsule (Solatene; Hoffmann-La Roche, Inc.) or matching placebos daily.

<sup>b</sup> IU, international units.

1983 cytology diagnosis of Dysplasia 1 were invited to participate in an endoscopic survey. Overall, 63% of those invited agreed to be endoscoped, including 461 originally diagnosed as Dysplasia 2 and 372 originally diagnosed as Dysplasia 1. Informed consent was obtained from each subject prior to the procedure.

During endoscopy, the entire esophagus and the stomach were visually examined and 1 or more 2.8-mm biopsies were taken from all focal lesions. If no focal lesions were found, a standard site in the mid-esophagus was sampled. No standard sampling of the stomach was made.

### 1991 Endoscopic Survey

In April and May, 1991, at the end of the 6-year Dysplasia Trial intervention, a second endoscopic survey was conducted. For logistical reasons, this survey was limited to Rencun commune, which did not differ significantly from the other two communes in age or sex distribution or esophageal/gastric cardia cancer rates. In 19 of the Rencun villages with the most trial participants, all subjects younger than 70-years old who had no history of cancer and had completed an end-of-trial cytology examination were invited to undergo endoscopy. Overall, 81% of those invited agreed to participate, and 396 subjects underwent endoscopy. Informed consent was obtained from each subject prior to the procedure.

During endoscopy, the entire esophagus and stomach were visually examined and 1 or more 2.8-mm biopsies were taken from all focal lesions and four standard sites (gastric angulus, cardia at 12:00, cardia at 6:00, and mid-esophagus).

### Histological Categories

The biopsy slides from both endoscopic surveys were read jointly by three pathologists (S. M. D., K. J. L., F. S. L.), without knowledge of the patient's history, treatment group, or the visual endoscopic findings. The histological criteria were based on previous descriptions (4-8).

**Esophageal Categories. Normal.** A stratified squamous epithelium was present which showed no features diagnostic of acanthosis, esophagitis, squamous dysplasia, or squamous cancer, as defined below.

**Acanthosis.** An otherwise normal epithelium was  $\geq 0.5$  mm thick.

**Esophagitis.** One or more of the following three criteria were present: (a) elongation of lamina propria papillae into the upper third of the epithelium together with basal cell hyperplasia, defined as a basal zone thickness  $>15\%$  of total epithelial thickness; (b) epithelial infiltration by neutrophils or eosinophils; or (c) a dense nonfollicular infiltrate of mononuclear inflammatory cells or neutrophils in the lamina propria.

**Squamous Dysplasia.** Nuclear atypia (enlargement, pleomorphism, and hyperchromasia), loss of normal cell polarity, and abnormal tissue maturation were present in the lower third (mild), in the lower two-thirds (moderate), or in all thirds (severe) of the epithelium, without invasion. Biopsies containing dysplastic cells which could not be graded because of biopsy size or orientation were categorized as squamous dysplasia, not otherwise specified.

**Squamous Cancer.** Neoplastic squamous cells were present which had invaded through the basement membrane.

**Gastric Categories. Normal.** A gastric mucosa was present which showed no features diagnostic of gastritis, gastric dysplasia, or adenocarcinoma, as defined below. No inflammatory infiltrate was allowed in normal biopsies from the gastric fundus or body, but a mild lymphoplasmacytic infiltrate was permitted in normal biopsies from the cardia or antrum.

**Gastritis without Atrophy.** Any inflammation other than a mild lymphoplasmacytic infiltrate in biopsies from the cardia or antrum was called gastritis. For the purposes of this study, we did not separate superficial from full-thickness involvement or chronic from chronic active inflammation. No atrophy (loss of glands) or metaplasia was identified.

**Atrophic Gastritis.** There was variable inflammation and loss of normal glands, with or without intestinal or pyloric metaplasia.

**Gastric Dysplasia.** Neoplastic features, including nuclear atypia and/or architectural abnormalities, were present but confined to the gastric epithelium, without invasion. Dysplasia was categorized as low grade or high grade based on the severity of the neoplastic features (8).

**Adenocarcinoma.** Neoplastic gastric epithelial cells were present which had invaded through the basement membrane.

Symptoms were ascertained by interview at the time of each endoscopic examination. In this article, we report on the prevalence of dysphagia, the most common symptom associated with cancer of the esophagus and gastric cardia. In 1987, subjects were asked, "Do you now have trouble swallowing?" (yes/no), while in 1991 they were asked, "Within the last year, how often have you experienced difficulty swallowing?" (daily, often, upon occasion, rarely/never).

### Analysis

Each endoscopic survey was analyzed separately, and analysis included all subjects who had at least one satisfactory biopsy. In 1987, 768 (92%) of the 833 endoscoped subjects had at least one satisfactory biopsy, including 754 (91%) with satisfactory esophageal biopsies and 86 (10%) with satisfactory gastric biopsies (69 with gastric cardia biopsies and 20 with biopsies from elsewhere in the stomach). In 1991, all 396 of the endoscoped subjects had at least one satisfactory biopsy, including 381 (96%) with satisfactory esophageal biopsies and 396 (100%) with satisfactory gastric biopsies (394 with gastric cardia biopsies and 381 with biopsies from elsewhere in the stomach). In both surveys, the percentage of subjects with satisfactory esophageal or gastric biopsies did not differ significantly by treatment group. All of the esophageal biopsies were squamous except for five biopsies in 1987 and two biopsies in 1991; these seven glandular esophageal biopsies were excluded from the analysis. All of the gastric biopsies in both surveys were glandular. In 1987, gastric atrophy was not recorded, so only one category of gastritis was used.

For each subject, a worst esophageal diagnosis (invasive cancer > dysplasia > esophagitis > acanthosis > normal) and a worst gastric diagnosis (invasive cancer > dysplasia > atrophic gastritis > gastritis without atrophy > normal) were determined. Then a worst overall diagnosis was derived to examine the overall effect of the nutritional supplements in a population in which both esophageal and gastric cardia cancers are significant causes of mortality: normal, a worst biopsy diagnosis of normal or acanthotic squamous mucosa or normal gastric mucosa; inflammation, esophagitis or gastritis; low-grade dysplasia, mild squamous dysplasia or low-grade gastric dysplasia; high-grade dysplasia, moderate or severe squamous dysplasia, squamous dysplasia not otherwise specified, or high-grade gastric dysplasia; and cancer, squamous cancer or adenocarcinoma. For each survey, the distributions of worst esophageal, worst gastric, and worst overall diagnoses were then compared by treatment group.

To exclude an influence of selection bias on our results, we determined that the number of subjects excluded from eligibility for the endoscopic examinations because of death or incident cancer, the refusal rates among eligibles, and the prevalence of dysphagia in the endoscoped subjects did not differ significantly by treatment group in either survey. In addition, the prevalence of dysphagia among those eligible for endoscopy did not differ between those who accepted and those who refused endoscopy in either survey.

Differences in the prevalence of risk factors between the total trial population and the 1987 or 1991 endoscopy cohorts and differences in risk factors between treatment groups in the endoscoped cohorts were tested with *t* tests for mean age and  $\chi^2$  tests for sex, smoking, alcohol use, and 1983 cytology diagnosis. Treatment group differences in the overall distribution of the esophageal, gastric, and worst overall biopsy diagnoses were also tested using  $\chi^2$  tests. OR<sup>3</sup> and CI for treatment effects were estimated using SAS PROC LOGIST (9) with adjustment for age, sex, smoking status, alcohol use, and 1983 cytology diagnosis.

### Results

In 1987, the mean age of endoscopy participants was 56 years. Forty-two % were males, 25% were smokers, 18% reported alcohol use, and 55% had a 1983 cytology diagnosis of Dysplasia 2. Compared with the total Dysplasia Trial population, this endoscopy cohort had fewer smokers (25 versus 29%; *P* = 0.031) and a larger proportion of subjects with a 1983 cytology diagnosis of Dysplasia 2 (55 versus 23%; *P* < 0.001). There was no significant difference in the prevalence of any of these characteristics between treatment groups in the endoscoped cohort.

In 1991, the mean age of the endoscoped subjects was 57 years. Forty-two % were males, 28% were smokers, 14% reported alcohol use, and 20% had a 1983 cytology diagnosis of Dysplasia 2. This endoscopy cohort was younger than the total trial population (mean age 57 versus 59 years in 1991; *P* < 0.001) and it had fewer subjects who used alcohol (14 versus 19%; *P* = 0.030). The only significant difference between treatment groups in this cohort was the presence of fewer drinkers in the placebo group (17 versus 24%; *P* = 0.034).

Cumulative pill disappearance rates, a measure of intervention compliance, were 98% for the endoscoped subjects in each treatment group in both 1987 and 1991. High compliance was also indicated by quarterly biochemical assessments of a sample of the total Dysplasia Trial population which showed significant improvements in blood levels of retinol, riboflavin, ascorbic acid, and  $\beta$ -carotene in the active treatment group throughout the intervention period (1).

Tables 2–4 show the esophageal, gastric, and worst overall biopsy diagnoses from the two endoscopy surveys, by treatment group. The overall distributions of these diagnoses were not significantly different by treatment group in either survey (*P* > 0.10 for all comparisons).

Table 2 shows the esophageal biopsy diagnoses. Both surveys had similar proportions of normal subjects in each treatment group. In 1987, there were fewer cases of dysplasia and cancer in the active treatment group than in the placebo group, but in 1991, subjects receiving active pills had more cancers.

In 1987, there were nine cases of gastric dysplasia, including seven from the cardia and two from elsewhere in the stomach, and 21 cases of gastric cancer, including 20 from the cardia. In 1991, there were also nine cases of gastric dysplasia, including eight from the cardia, and 37 cases of gastric cancer, including 33 from the cardia. In both surveys, treatment group comparisons of the distributions of diagnoses were similar for the cardia and noncardia gastric biopsies, so only the combined data are shown. Table 3 shows the gastric biopsy diagnoses from both years. The two surveys had similar proportions of normal subjects. In 1987, the active treatment group had fewer cases of gastritis and more cases of dysplasia and cancer than the placebo group, but in 1991, these differences were not found.

Table 4 shows the worst overall diagnoses in the two surveys. In each year, the distribution of diagnoses was similar in each treatment group.

Odds ratios for those taking active supplements (versus those taking placebo pills) having a diagnosis of dysplasia or cancer are shown in Table 5. The odds ratios for esophageal lesions were below 1.0 in 1987 and near or above 1.0 in 1991, while for gastric lesions they were above 1.0 in 1987 and below 1.0 in 1991. Only the reduced risk of esophageal dysplasia or cancer in 1987 (OR, 0.74; 95% CI, 0.54–1.03; *P* = 0.073) and the increased risk of gastric dysplasia or

<sup>3</sup> The abbreviations used are: OR, odds ratio; CI, confidence interval.

Table 2 Linxian Dysplasia Trial endoscopic surveys esophageal biopsy results

Treatment group	No. of subjects endoscoped <sup>a</sup>	Worst esophageal diagnosis								Cancer
		Normal	Acanthosis	Esophagitis	Dysplasia				Total	
					Mild	Mod <sup>b</sup>	Sev <sup>b</sup>	NOS <sup>b</sup>		
1987 Survey										
Active	392	223 (56.9) <sup>c</sup>	52 (13.3)	21 (5.4)	36 (9.2)	17 (4.3)	23 (5.9)	4 (1.0)	80 (20.4)	16 (4.1)
Placebo	362	203 (56.1)	35 (9.7)	14 (3.9)	44 (12.2)	18 (5.0)	21 (5.8)	8 (2.2)	91 (25.1)	19 (5.3)
Total	754	426 (56.5)	87 (11.5)	35 (4.6)	80 (10.6)	35 (4.6)	44 (5.8)	12 (1.6)	171 (22.7)	35 (4.6)
1991 Survey										
Active	195	134 (68.7)	17 (8.7)	13 (6.7)	10 (5.1)	4 (2.1)	9 (4.6)		23 (11.8)	8 (4.1)
Placebo	186	127 (68.3)	10 (5.4)	19 (10.2)	17 (9.1)	3 (1.6)	5 (2.7)		25 (13.4)	5 (2.7)
Total	381	261 (68.5)	27 (7.1)	32 (8.4)	27 (7.1)	7 (1.8)	14 (3.7)		48 (12.6)	13 (3.4)

<sup>a</sup> Fourteen subjects had no satisfactory squamous biopsies in 1987 and 15 subjects had no satisfactory squamous biopsies in 1991.

<sup>b</sup> Mod, moderate dysplasia; Sev, severe dysplasia; NOS, dysplasia not otherwise specified.

<sup>c</sup> Number of subjects (row %).

Table 3 Linxian Dysplasia Trial endoscopic surveys gastric biopsy results

1987 Survey										
Treatment group	No. of subjects endoscoped <sup>a</sup>	Worst gastric biopsy diagnosis						Cancer		
		Normal	Gastritis	Dysplasia			Total			
				Low grade	High grade	Total				
Active	43	2 (4.7) <sup>b</sup>	22 (51.2)	4 (9.3)	2 (4.7)	6 (14.0)	13 (30.2)			
Placebo	43	2 (4.7)	30 (69.8)	1 (2.3)	2 (4.7)	3 (7.0)	8 (18.6)			
Total	86	4 (4.7)	52 (60.5)	5 (5.8)	4 (4.7)	9 (10.5)	21 (24.4)			
1991 Survey										
Treatment group	No. of subjects endoscoped	Worst gastric biopsy diagnosis						Cancer		
		Normal	Gastritis without atrophy	Atrophic gastritis	Dysplasia				Total	
					Low grade	High grade	Total			
Active	202	52 (25.7) <sup>b</sup>	76 (37.6)	52 (25.7)	2 (1.0)	2 (1.0)	4 (2.0)	18 (8.9)		
Placebo	194	52 (26.8)	65 (33.5)	53 (27.3)	4 (2.1)	1 (0.5)	5 (2.6)	19 (9.8)		
Total	396	104 (26.3)	141 (35.6)	105 (26.5)	6 (1.5)	3 (0.8)	9 (2.3)	37 (9.3)		

<sup>a</sup> Six hundred eighty-two subjects had no satisfactory gastric biopsies in 1987.

<sup>b</sup> Number of subjects (row %).

cancer in 1987 (OR, 2.49; 95% CI, 0.94–6.58;  $P = 0.066$ ) approached statistical significance. The odds ratio for subjects in the treatment group having a worst overall diagnosis of dysplasia or cancer was 0.84 (95% CI, 0.61–1.15) in 1987 and 0.86 (95% CI, 0.54–1.38) in 1991.

In both surveys, nearly all of the endoscoped subjects who were interviewed were asymptomatic with respect to dysphagia, the primary symptom of esophageal and gastric cardia cancers. In 1987, only 22/744 (3.0%) reported difficulty swallowing (yes versus no), including 4 of 166 (2.4%) with esophageal dysplasia, 3 of 35 (8.6%) with esophageal cancer, 0 of 8 with gastric dysplasia, and 0 of 21 with gastric

cancer. In 1991, only 3/393 (0.8%) reported dysphagia (daily or often versus occasional or rare/never), including 0 of 48 with esophageal dysplasia, 1 of 13 (7.7%) with esophageal cancer, 0 of 9 with gastric dysplasia, and 1 of 37 (2.7%) with gastric cancer.

## Discussion

The 1987 and 1991 endoscopy examinations were point prevalence surveys of early neoplastic lesions in asymptomatic individuals with no history of cancer. These studies complement the overall cancer incidence and mortality re-

Table 4 Linxian Dysplasia Trial endoscopic surveys worst overall biopsy results

Cancer	Treatment group	No. of subjects endoscoped	Worst overall biopsy diagnosis					Cancer
			Normal	Inflammation	Dysplasia		Total	
					Low grade	High grade		
	1987 Survey							
16 (4.1)	Active	400	245 (61.3) <sup>a</sup>	40 (10.0)	39 (9.8)	47 (11.8)	86 (21.5)	29 (7.3)
19 (5.3)	Placebo	368	212 (57.6)	37 (10.1)	43 (11.7)	49 (13.3)	92 (25.0)	27 (7.3)
35 (4.6)	Total	768	457 (59.5)	77 (10.0)	82 (10.7)	96 (12.5)	178 (23.2)	56 (7.3)
	1991 Survey							
8 (4.1)	Active	202	36 (17.8)	119 (58.9)	10 (5.0)	14 (6.9)	24 (11.9)	23 (11.4)
5 (2.7)	Placebo	194	40 (20.6)	104 (53.6)	17 (8.8)	9 (4.6)	26 (13.4)	24 (12.4)
13 (3.4)	Total	396	76 (19.2)	223 (56.3)	27 (6.8)	23 (5.8)	50 (12.6)	47 (11.9)

<sup>a</sup> Number of subjects (row %).

Table 5 Linxian Dysplasia Trial endoscopic surveys odds ratios for active treatment on esophageal and gastric dysplasia and cancer

Cancer	Survey	Esophageal diagnoses				Gastric diagnoses				Worst overall diagnoses			
		Dysplasia or cancer		Cancer		Dysplasia or cancer		Cancer		Dysplasia or cancer		Cancer	
		n <sup>a</sup>	OR <sup>b</sup> (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)
	1987	205 <sup>c</sup>	0.74 (0.54-1.03)	35	0.79 (0.40-1.57)	30	2.49 (0.94-6.58)	21	1.91 (0.64-5.68)	233 <sup>c</sup>	0.84 (0.61-1.15)	56	1.02 (0.58-1.78)
13 (30.2)	1991	61	1.01 (0.58-1.76)	13	1.49 (0.47-4.72)	46	0.77 (0.41-1.47)	37	0.77 (0.38-1.58)	97	0.86 (0.54-1.38)	47	0.79 (0.42-1.51)

<sup>a</sup> Number of subjects with the specified diagnosis.

<sup>b</sup> Odds ratios, adjusted for age, gender, smoking, alcohol use and 1983 cytology diagnosis, with 95% confidence limits.

<sup>c</sup> One case of esophageal dysplasia deleted due to missing covariate information.

sults of the Dysplasia Trial (2), which included predominantly symptomatic, later stage tumors. The 14-16% reductions in early esophageal and gastric lesions reported here are consistent with the 8% lower risk for esophageal and gastric cardia cancer death which was found in the supplemented group of the total trial during the 6-year intervention.

Although the results of the worst overall diagnoses were consistent between surveys and paralleled the mortality results from the trial as a whole, the site-specific effects of supplementation gave somewhat different results in the two endoscopic examinations. For both esophageal and gastric biopsies, there were opposite point estimates for treatment effects in 1987 and 1991, with the 1987 results more in accord with the mortality results from the total trial. The most likely reason for these differences was estimate instability due to small numbers of subjects, but other dissimilarities between the surveys, such as the difference in duration of supplementation, the difference in prevalence of subjects with high-grade cytologic dysplasia, and the difference in gastric sampling methods may also have contributed. None of the estimates of effect in either survey differed significantly from the null value of 1.

Several factors may have limited the ability of our endoscopic examinations to demonstrate treatment effects. The nutrients may have been given for an insufficient length of time, in insufficient doses, or too late in the lives of the subjects for an effect to be seen. Relevant nutrients may have

been omitted (although this seems unlikely, given the large number of vitamins and minerals included), or protective effects of some nutrients may have been balanced by harmful effects of others. In addition, although our endoscopic surveys were among the largest reported from high-risk areas of China, relatively few dysplasia and cancer events were identified, so small but worthwhile treatment benefits may not have been detected. Small sample size is a common limitation in endoscopic surveys because of the invasiveness of the procedure and the large amount of time and work required for each subject.

All of the subjects in both endoscopic surveys had a cytological diagnosis of dysplasia in 1983, including 98% with a diagnosis of squamous dysplasia. But only 27% (206/754) of those endoscoped in 1987 and 16% (61/381) of those examined in 1991 were found to have squamous dysplasia or squamous carcinoma in their endoscopic biopsies. This discrepancy may be due to a combination of factors, including regression of dysplastic lesions in the 4-8 years between the cytological and endoscopic examinations, incomplete identification of squamous dysplasia and cancer by the endoscopic biopsies, and false positive initial cytological diagnoses. The ability of the biopsy protocols used in these surveys to identify most foci of squamous dysplasia and cancer is supported by recent studies showing that most of these histologic abnormalities in Linxian are associated with endoscopically visible focal lesions similar to the ones

targeted in 1987 and 1991 (10) and that the 1987 protocol achieved a high correlation between biopsy diagnoses of squamous dysplasia and subsequent development of invasive squamous cancer.<sup>4</sup> With regard to the cytological diagnoses, it should be noted that the Chinese cytological categories and criteria used in the 1983 screening (3) have not yet been carefully compared with the cytological categories used in other countries or with same-site biopsy diagnoses, but their ability to identify individuals with increased risk for future esophageal and gastric cardia cancer has been documented (11).<sup>5</sup>

Two previous studies have reported squamous esophageal biopsy results after prospective randomized trials of nutritional supplements in high-risk Chinese populations (12, 13). In one study (12), 610 subjects were randomized between supplementation with riboflavin (200 mg/week), retinol (50,000 international units/week) and zinc (50 mg/week) and placebo for 13.5 months. In the other study (15), 200 subjects were randomized between calcium supplementation (1200 mg/day) and placebo for 11 months. Neither study found a significant difference in the distribution of biopsy diagnoses between the treatment and placebo groups. Both studies may have been limited by their few cases of squamous dysplasia or cancer. Even with larger numbers of such cases, however, our results were still inconclusive. To our knowledge, there are no previous reports of gastric biopsy surveys after randomized nutritional intervention trials.

In summary, although modest protective effects on worst overall diagnosis were seen in the supplemented group in both surveys, none of the results was statistically significant, and the findings must be considered inconclusive. It is likely that longer interventions and larger numbers of endoscoped subjects will be required to fully investigate the effects of micronutrient supplementation in this population.

<sup>4</sup> S. M. Dawsey, K. J. Lewin, G. Q. Wang, F. S. Liu, R. K. Nieberg, Y. Yu, J. Y. Li, W. J. Blot, B. Li, and P. R. Taylor, Squamous esophageal histology and subsequent risk of squamous esophageal cancer: a prospective follow-up from Linxian, China, submitted for publication.

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