

Prevention of Esophageal Cancer: The Nutrition Intervention Trials in Linxian, China¹

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Abstract

In Linxian China, the esophageal/gastric cardia cancer mortality rates are among the highest in the world. There is suspicion that the population's chronic deficiencies of multiple micronutrients are etiologically involved. We conducted two randomized, placebo-controlled nutrition intervention trials to test the effects of vitamin and mineral supplements in lowering the rates of esophageal/gastric cancer. In the first trial, the dysplasia trial, 3318 adults with a cytological diagnosis of esophageal dysplasia received daily supplementation with 26 vitamins and minerals in doses typically 2–3 times the United States Recommended Daily Allowances, or placebos, for 6 years. The second trial, the general population trial, involved 29,584 adults and used a one-half replicate of a 2⁴ factorial experimental design which tested the effects of four combinations of nutrients: A, retinol and zinc; B, riboflavin and niacin; C, vitamin C and molybdenum; and D, β -carotene, vitamin E, and selenium. Doses for these daily supplements ranged from 1 to 2 times the United States Recommended Daily Allowances, and the different vitamin/mineral combinations or placebos were taken for a period of 5.25 years. As part of the general population trial, and end-of-intervention endoscopy survey was carried out in a small (1.3%) sample of subjects to see if supplementation affected the prevalence of dysplasia and early cancer. Herein we review the methods of these trials and the results of the endoscopic survey. Fifteen esophageal and 16 gastric cancers were identified in endoscopic biopsies from the 391 subjects evaluated from two villages, and nearly all were asymptomatic. No significant reductions in the prevalence of esophageal or gastric dysplasia or cancer were seen with any of the four supplement groups. However, the prevalence of gastric cancer among participants receiving retinol and zinc was 62% lower than those not receiving those supplements ($P = 0.09$), while participants receiving β -carotene, vitamin E, and selenium had a 42% reduction in esophageal cancer prevalence (0.34). We have reported separately that cancer mortality over the entire 5.25-year period was significantly reduced among those receiving β -carotene, vitamin E, and selenium. The findings from the overall trial and the endoscopic sample offer a hopeful sign and should encourage additional studies with these agents in larger numbers of subjects.

Introduction

Some of the world's highest incidence and mortality rates for cancer of the esophagus occur in Linxian, China (1). The excess cancers occur not only as squamous cell carcinomas of the esophagus, but also as adenocarcinomas of the gastric cardia. Traditionally, both of these tumors have been called "esophageal cancer" in Linxian,

because of their proximity to one another and their similarity in symptoms. The reasons for the exceptionally high cancer rates in Linxian are not known, but the population's chronic deficiencies of multiple micronutrients are one likely cause (2–9). To test whether supplementation with vitamins and minerals could reduce cancer rates, two randomized, placebo-controlled trials were conducted (10). The first, the dysplasia trial, enrolled individuals with cytologically diagnosed esophageal dysplasia while the second, the general population trial, enrolled individuals from the general population of this high-risk area. Both trials evaluated the effects of multiple vitamin and mineral supplementation on esophageal/gastric cardia cancer incidence and mortality as well as mortality from other causes. At the end of the trials, an endoscopic survey was conducted in a sample of subjects to see if supplementation had affected the prevalence of dysplasia or early cancer. The current report reviews the methods of both trials and gives results from the end-of-intervention endoscopic survey in the general population trial.

Methods

Dysplasia Trial. The dysplasia trial, conducted in 3 communes (Yaocun, Rencun, and Donggang) in northern Linxian, enrolled 40- to 69-year-old adults who had evidence of esophageal dysplasia on a balloon cytology examination but no history of malignancy. These subjects were randomized to receive multiple vitamin/mineral supplements or placebos daily for a period of 6 years (11). The micronutrients provided in the supplements included 14 vitamins and 12 minerals (*i.e.*, β -carotene as Solatene, Hoffmann-La Roche, Inc., and multivitamins as Centrum, Lederle Laboratories, Inc.)⁴ in doses typically 2–3 times the United States Recommended Daily Allowances (12).

A total of 3318 persons were randomized and started intervention in May of 1985. The median age of participants at the start of intervention was 54 years, 56% were female, 29% smoked tobacco, 18% reported alcohol use the past year, and 43% had a family history of esophageal or stomach cancer. The treatment groups were similar for all characteristics examined except that more participants in the supplement group had dysplasia 2 (high-grade dysplasia) on their pretrial cytology examination than in the placebo group (25 versus 22%).

Village doctors distributed pills monthly throughout the trial. Compliance was assessed by counting all residual pills monthly and by assaying nutrient levels in blood on a sample of persons each quarter. The overall pill disappearance rate was 94% in both treatment groups and the blood assays showed significantly greater increases in nutrient status for persons in the supplement group (*e.g.*, serum β -carotene levels rose 10-fold in the supplement group compared with an 11% rise in the placebo group).

Incident cancers and deaths were identified through several methods that assured essentially complete ascertainment of events. These included

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⁴ Micronutrients and their total daily dose were: β -carotene (15 mg); vitamin A (10,000 IU); vitamin E (60 IU); vitamin C (180 mg); folic acid (800 μ g); vitamin B₁ (5 mg); vitamin B₂ (5.2 mg); niacinamide (40 mg); vitamin B₆ (6 mg); vitamin B₁₂ (18 μ g); vitamin D (800 IU); biotin (90 μ g); pantothenic acid (20 mg); calcium (324 mg); phosphorus (250 mg); iodine (300 μ g); iron (54 mg); magnesium (200 mg); copper (6 mg); manganese (15 mg); potassium (15.4 mg); chloride (14 mg); chromium (30 μ g); molybdenum (30 μ g); selenium (50 μ g); zinc (45 mg).

monthly visits by village doctors, review of local and regional hospital records and the local cancer registry, and cytology and endoscopy surveys performed during the trial. A total of 448 incident cancers were identified during the trial (13.5% of the total trial population), including 251 esophageal and 159 gastric cardia cancers; 324 deaths occurred during the intervention of which 54% were due to cancer.

General Population Trial. The general population trial, conducted in 4 communes (Yaocun, Rencun, Donggang, and Hengshui) in northern Linxian, enrolled 40- to 69-year-old adults from the general population with no history of malignancy. These subjects were randomized to intervention groups receiving daily vitamin/mineral supplementation by using a one-half replicate of a 2⁴ factorial experimental design for a period of 5.25 years (13). Table 1 shows the fractional factorial design used. This design enabled simultaneous testing for the effects of four different combinations of nutrients or treatment factors. Nutrients and doses, shown in Table 2, ranged from 1 to 2 times the United States Recommended Daily Allowances (12).

A total of 29,584 persons were randomized and started intervention in March of 1986. The median age of participants at the start of intervention was 52 years, 55% were female, 30% smoked tobacco, 23% reported alcohol use the past year, and 32% had a family history of esophageal or stomach cancer. Treatment groups were similar for all characteristics examined.

As with the dysplasia trial, each month village doctors distributed new pills and counted residual ones, and each quarter a sample of persons had nutrient levels assayed in blood. The overall pill disappearance rate was 93% and did not differ by intervention group. Likewise, nutrient assays confirmed compliance for each of the four different treatment factors (e.g., serum β-carotene levels rose 14-fold in persons receiving this nutrient compared with a less than 2-fold rise in persons not receiving it).

Incident cancers and deaths were identified in the general population trial in a manner similar to the dysplasia trial and ascertainment was considered essentially complete. A total of 1298 incident cancers were identified during the trial (4.4% of the total trial population), including 640 esophageal and 435 gastric cardia cancers. Over 2100 deaths occurred during the intervention, of which 37% were due to cancer.

General Population Trial Endoscopy Survey. At the end of the general population trial intervention, an endoscopic survey was carried out to assess the effect of the intervention factors on the distribution of biopsy findings (14). All trial participants from two of the

Table 1 Fractional factorial design used in the general population trial in Linxian, China^a

Placebo	A	B	AB
C	AC	BC	ABC
D	AD	BD	ABD
CD	ACD	BCD	ABCD

^a 2⁴ factorial design has all 16 combinations shown. The fractional factorial design used only the 8 combinations italicized.

Table 2 Daily doses and types of micronutrients by treatment factor in the general population trial, Linxian, China

Factor	Micronutrients	Dose/day
A	Retinol (as palmitate) Zinc (as zinc oxide)	5000 IU 22.5 mg
B	Riboflavin Niacin	3.2 mg 40 mg
C	Ascorbic acid Molybdenum (as molybdenum yeast complex)	120 mg 30 μg
D	β-Carotene Selenium (as selenium yeast) α-Tocopherol	15 mg 50 μg 30 mg

Table 3 Esophageal biopsy results from general population trial 1991 endoscopic survey

Treatment factor ^a	No. of subjects ^b	Worst esophageal biopsy diagnosis (%)			
		Normal or acanthosis	Esophagitis	Dysplasia	Cancer
A	192	83	6	7	4
No A	187	82	8	6	4
B	188	82	7	7	4
No B	191	83	7	6	4
C	201	82	7	7	4
No C	178	83	7	6	3
D	173	85	5	7	3
No D	206	80	9	6	5

^a See Table 2 for doses and types of micronutrients in each factor.

^b Twelve subjects had no satisfactory esophageal biopsies.

Table 4 Odds ratios (95% confidence intervals) for treatment effects on esophageal dysplasia and cancer in the general population trial endoscopic survey^a

Treatment factor ^b	Esophageal diagnoses	
	Dysplasia or cancer (N = 39 cases)	Cancer (N = 15 cases)
A (vs. no A)	1.12 (0.57–2.20)	1.02 (0.36–2.91)
B (vs. no B)	1.12 (0.58–2.19)	1.19 (0.42–3.39)
C (vs. no C)	1.31 (0.67–2.57)	1.32 (0.46–3.83)
D (vs. no D)	0.80 (0.40–1.57)	0.58 (0.19–1.76)

^a Odds ratios adjusted for age, gender, smoking, and alcohol use.

^b See Table 2 for doses and types of micronutrients in each factor.

largest villages in Rencun commune who were alive, less than 70 years of age, without a prior diagnosis of cancer, and who had also participated in an end-of-trial cytology examination were invited. Endoscopic examination consisted of complete visualization of the esophagus and stomach, followed by biopsies of all worrisome areas and lesions and four standard-site biopsies [gastric angulus, cardia (2), and midesophagus]. The end points for the survey were the worst squamous histology from the esophagus, the worst glandular histology from the stomach, and the worst overall histology from any site. Multiple logistic regression was used to estimate the relative prevalence of these end points according to intervention group following adjustment for age, gender, smoking, and alcohol use.

Results

Three hundred ninety-one trial participants (79% of those eligible) from two villages targeted for the survey were endoscoped at the end of the general population trial. Their median age at the start of the intervention was 48 years, 50% were female, 36% smoked cigarettes, and 37% reported alcohol use. There were no significant differences by treatment factor for any of these characteristics. Cumulative pill disappearance rates were 96–97% for the endoscoped subjects in each factor group.

Table 3 shows distribution of esophageal histology results by treatment factor. Overall, the worst esophageal diagnosis for 82% of the participants was normal or acanthosis, 7% had esophagitis, 7% had dysplasia, and 4% had cancer. All but 2 of the 39 subjects with dysplasia or cancer were asymptomatic. No striking differences by treatment group were observed. Table 4 shows the odds ratios for the four treatment effects for the combined end point of esophageal dysplasia plus cancer and esophageal cancer alone. Risk estimates for factor D (β-carotene/vitamin E/selenium) were less than one, with a 42% reduction in risk for esophageal cancer, but this result did not approach statistical significance.

Table 5 shows the distribution of gastric histology results by treatment factor. Overall, 35% of participants had a worst gastric histology than was normal, 59% had gastritis, 2% showed dysplasia, and 4% had cancer. All 7 dysplasias and 14 of 16 of the cancers were

Table 5 Gastric biopsy results from the general population trial 1991 endoscopic survey

Treatment factor ^a	No. of subjects ^b	Worst gastric biopsy diagnosis (%)			
		Normal	Gastritis	Dysplasia	Cancer
A	196	35	60	2	3
No A	194	35	58	2	6
B	193	35	58	2	5
No B	197	35	60	2	3
C	205	36	57	2	6
No C	185	35	62	1	2
D	176	32	63	1	4
No D	214	37	56	2	4

^a See Table 2 for doses and types of micronutrients in each factor.

^b One subject had no satisfactory gastric biopsies.

Table 6 Odds ratios (95% confidence intervals) for treatment effects on gastric dysplasia and cancer in the general population trial endoscopic survey^a

Treatment factor ^b	Gastric diagnoses	
	Dysplasia or cancer (N = 23 cases)	Cancer (N = 16 cases)
A (vs. no A)	0.58 (0.24–1.39)	0.38 (0.13–1.15)
B (vs. no B)	1.32 (0.42–3.39)	1.67 (0.58–4.76)
C (vs. no C)	2.64 (1.01–6.93)	2.75 (0.86–8.84)
D (vs. no D)	0.83 (0.37–2.01)	1.05 (0.37–2.92)

^a Odds ratios adjusted for age, gender, smoking, and alcohol use.

^b See Table 2 for doses and types of micronutrients in each factor.

found in the gastric cardia. All 23 subjects with these malignant or premalignant lesions were asymptomatic. Odds ratios for treatment effects on gastric end points are shown in Table 6. Factor A (retinol/zinc) reduced gastric dysplasia or cancer by 42% and cancer alone by 62%, although neither of these reductions was significant. Risks were increased for factors B and C, while factor D showed a slight reduction in dysplasia or cancer risk.

When esophageal and gastric biopsies were combined for each subject into a worst overall diagnosis, factor A showed a reduction in dysplasia or cancer (odds ratios = 0.83, 95% confidence intervals = 0.47–1.46, based on 59 cases) and cancer (0.61, 0.29–1.31, based on 31 cases), factors B and C showed modest increases in risk for these end points, and factor D showed reduced risks for dysplasia or cancer (odds ratios = 0.83, 95% confidence intervals = 0.47–1.46), and cancer (0.79, 0.36–1.69).

Discussion

Two randomized, nutrition intervention trials have been conducted in Linxian, China, to test whether 5–6 years of daily supplements with vitamin/mineral combinations can reduce the incidence and mortality of esophageal/gastric cardia cancer. As part of these trials, an end-of-intervention endoscopic survey was performed in a sample of subjects to evaluate the prevalence of early neoplastic disease.

The endoscopic survey conducted at the conclusion of the general population trial resulted in several interesting observations. First, the prevalence of cancer, 4% esophageal cancer and 4% gastric cardia cancer, or 8% overall, was extraordinary. Second, nearly all the gastric cancers and all cases of gastric dysplasia were found in the cardia. Finally, nearly all persons with either dysplasia or cancer of the esophagus or stomach had early lesions and were asymptomatic with regard to dysphagia, the primary symptom of esophageal and gastric cardia cancers.

Although no significant reductions in the prevalence of dysplasia or cancer were observed in this endoscopic survey after 5.25 years of nutrient supplementation, suggestive benefits on early lesions were seen, retinol/zinc reduced early gastric cardia risk, β -carotene/vitamin

E/selenium reduced risk of early esophageal cancer, and both of these intervention factors resulted in modest reductions in overall risk.

There are a number of limitations that need to be considered in interpreting these findings. The number of participants endoscoped was relatively small and the number of end points even smaller; the observed estimates of treatment effects were generally modest and somewhat unstable; and bias is always a potential problem when evaluating a selected subgroup as was done here.

We have separately reported that cancer mortality over the 5.25-year intervention was significantly lower in the approximately 15,000 individuals who received daily supplementation with β -carotene, vitamin E, and selenium (13). While no significant protective effects were seen for any of the 4 vitamin/mineral supplements in the smaller endoscopic survey, the findings are promising. Additional studies with larger numbers of end points are needed to further evaluate their potential.

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