

# The Linxian trials: mortality rates by vitamin-mineral intervention group<sup>1,2</sup>

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**ABSTRACT** Two randomized nutrition intervention trials were conducted in Linxian, an area of northcentral China with some of the world's highest rates of esophageal and stomach cancer and a population with a chronically low intake of several nutrients. One trial used a factorial design that allowed us to assess the effects in nearly 30 000 participants of daily supplementation with four nutrient combinations: retinol and zinc; riboflavin and niacin; vitamin C and molybdenum; and  $\beta$ -carotene,  $\alpha$ -tocopherol, and selenium. The second trial provided daily multiple vitamin-mineral supplementation or placebo in 3318 persons with esophageal dysplasia, a precursor to esophageal cancer. After supplements were given for 5.25 y in the general population trial, small but significant reductions in total [relative risk (RR) = 0.91] and cancer (RR = 0.87) mortality were observed in subjects receiving  $\beta$ -carotene,  $\alpha$ -tocopherol, and selenium but not the other nutrients. The reductions were greater in women than men, and in those under compared with over the age of 55; however, differences by sex or age were not significant. After multiple vitamin and mineral supplements were given for 6 y in the smaller dysplasia trial, reductions in total (RR = 0.93) and cancer (RR = 0.96) mortality were observed but these were not significant. The largest reductions were for cerebrovascular disease mortality, but the effects differed by sex: a significant reduction was observed in men (RR = 0.45) but not women (RR = 0.90). Restoring adequate intake of certain nutrients may help to lower the risk of cancer and other diseases in this high-risk population. *Am J Clin Nutr* 1995; 62(suppl):1424S-6S.

**KEY WORDS** Esophageal cancer, stomach cancer, vitamins, minerals,  $\beta$ -carotene, vitamin E, selenium, mortality, randomized trials

## INTRODUCTION

Some of the world's highest rates of cancer occur in Linxian, a rural county in northcentral China (1). Tumors primarily affect the esophagus and gastric cardia, with > 85% of all malignancies appearing in these anatomic sites. Because esophageal and stomach cancers are known through epidemiologic studies around the world to be influenced by diet and nutrition (2) and because the Linxian population historically has a low intake of several nutrients (although frank clinical deficiencies are rare), the population seemed ideal for a trial to test whether improving nutritional status through vitamin and mineral supplementation would lower cancer risk. Thus, two trials were launched in the mid-1980s. We summarize results

from the trials, presenting for the first time relative risks associated with the supplements according to sex and age.

## METHODS

Detailed descriptions of the methods of the intervention trials are presented elsewhere (3–5). In brief, in one trial, the general population trial, 29 584 adults aged 40–69 from four Linxian communes were randomly assigned into eight groups. The groups were specified by a fractional factorial design used to test the effects of four different vitamin-mineral combinations (Table 1). Supplementation began in March 1986 and continued through April 1991. In the second trial, the dysplasia trial, 3318 adults aged 40–69 y from three of these communes were randomly assigned into two groups: one to receive a multiple vitamin and mineral supplement (Table 2), the other a look-alike placebo. The individuals had been diagnosed with esophageal dysplasia before the start of the trial, primarily via mass balloon cytology screening campaigns initiated to detect early esophageal cancers. Supplementation began in May 1985 and ended in April 1991. Compliance in both trials was assessed by counting unused pills and through quarterly biochemical assessment of serum nutrient concentrations in randomly selected participants.

All deaths and all incident cancers in trial participants were ascertained through routine and special follow-up that ensured essentially complete reporting of these events. The cancer (but not other) diagnoses were reviewed by an expert panel of Chinese and American physicians skilled in the diagnosis of esophageal and gastric cancer. In the fall of 1987 and spring of 1991, participants in the dysplasia trial were offered repeat cytologic examinations for detection of early cancers and precancerous lesions.

Relative risks (RRs) of mortality from cancer, cerebrovascular disease, and other causes were determined for those who did versus those who did not receive each of the four vitamin and mineral combinations in the general population trial and for those who did versus those who did not receive the multiple vitamin and mineral supplements in the dysplasia trial. Proportional hazards regression methods (6) were used to estimate the

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TABLE 1

Nutrient combinations and doses used in the general population trial

Factor and micronutrients	Dose per day
Supplement A	
Retinol (as palmitate; IU)	5000
Zinc (as zinc oxide; mg)	22.5
Supplement B	
Riboflavin (mg)	3.2
Niacin (mg)	40
Supplement C	
Ascorbic acid (mg)	120
Molybdenum (as molybdenum yeast complex; $\mu\text{g}$ )	30
Supplement D	
$\beta$ -Carotene (mg)	15
Selenium (as selenium yeast; $\mu\text{g}$ )	50
$\alpha$ -Tocopherol (mg)	30

RR and corresponding 95% CI. The models included adjustments for cigarette smoking (yes or no), family history of cancer (yes or no), severity of dysplasia (grade 2 or grade 1) for dysplasia trial participants, and age or sex or both. Models for men and women separately omitted the term for sex, whereas models for those older than versus those younger than 55 y retained a term for year of age.

## RESULTS

Among participants of the general population and dysplasia trials, 2127 and 324 deaths occurred, respectively (Table 3). Cancer was the leading cause of death. The tumors were primarily esophageal and stomach (mostly gastric cardia) cancers, with few lung or other cancers. Cerebrovascular disease was the second leading cause of death.

We have previously reported (4) that mortality from cancer was significantly lower for individuals in the general population trial who received the  $\beta$ -carotene, vitamin E, and selenium supplements. Table 4 shows that the reduction in cancer mortality was greater in women (RR = 0.79, 95% CI = 0.64, 0.98) than men (RR = 0.93, 95% CI = 0.77, 1.12) and among those less than (RR = 0.71, 95% CI = 0.55, 0.92) compared with those equal to or greater than (RR = 0.94, 95% CI = 0.80, 1.11) the age of 55 at the start of intervention. However, the CIs for these subgroups overlap, and the sex ( $P = 0.40$ ) and age ( $P = 0.09$ ) differences are not statistically significant.

There was smaller variation in cancer death rates by sex or age in the dysplasia trial (Table 5), although the RRs for cancer and mortality for those receiving multiple vitamin and mineral supplementation also were lower in women and in those aged < 55 y. There was a marked difference ( $P = 0.21$ ) by sex in risk of cerebrovascular disease mortality: taking the supplements was associated with a significantly reduced rate of cerebrovascular mortality among men (RR = 0.45, 95% CI = 0.20, 0.98), but not women (RR = 0.90, 95% CI = 0.43, 1.92). This effect of the supplementation appeared greater at older ages, although this age difference was not significant ( $P = 0.38$ ).

## DISCUSSION

The Lixian intervention trials provide evidence that nutritional supplementation during adulthood may lower the risk of

TABLE 2

Daily doses and types of micronutrients used in the dysplasia trial<sup>1</sup>

Vitamin or mineral and compound	Dose
$\beta$ -Carotene (acetate; mg)	15
Vitamin A (acetate; IU)	10 000
Vitamin E (2- <i>ambo</i> - $\alpha$ -tocopherol; IU)	60
Vitamin C (ascorbic acid; mg)	180
Folic acid ( $\mu\text{g}$ )	800
Vitamin B-1 (thiamine mononitrate; mg)	5
Vitamin B-2 (riboflavin; mg)	5.2
Niacinamide (mg)	40
Vitamin B-6 (pyridoxine HCl; mg)	6
Vitamin B-12 (cyanocobalamin; $\mu\text{g}$ )	18
Vitamin D (IU)	800
Biotin ( $\mu\text{g}$ )	90
Pantothenic acid (calcium pantothenate; mg)	20
Calcium (dibasic calcium phosphate; mg)	324
Phosphorus (dibasic calcium phosphate; mg)	250
Iodine (potassium iodide; $\mu\text{g}$ )	300
Iron (ferrous fumarate; mg)	54
Magnesium (magnesium oxide; mg)	200
Copper (cupric oxide; mg)	6
Manganese (manganese sulfate; mg)	15
Potassium (potassium chloride; mg)	15.4
Chloride (potassium chloride; mg)	14
Chromium (chromium chloride; $\mu\text{g}$ )	30
Molybdenum (sodium molybdate; $\mu\text{g}$ )	30
Selenium (sodium selenate; $\mu\text{g}$ )	50
Zinc (zinc sulfate; mg)	45

<sup>1</sup> Participants received two multivitamin, multiminerals tablets (Centrum; Lederle Laboratories, Wayne, NJ) and one  $\beta$ -carotene capsule (Solatene; Hoffmann-LaRoche, Nutley, NJ) or matching placebos daily.

cancer (primarily of the esophagus and gastric cardia) in this high-risk population. A modest but significant reduction in cancer mortality was seen in the general population trial in those receiving daily a combination of  $\beta$ -carotene, vitamin E, and selenium, whereas no appreciable effects were found for the other types of supplements. In the smaller dysplasia trial, the most pronounced effect was the lower risk of cerebrovascular disease mortality among those who received the multiple vitamin and mineral supplements.

The analyses of sex- and age-specific RRs presented in this report indicate that the reduction in cancer mortality associated with  $\beta$ -carotene, vitamin E, and selenium supplementation was more pronounced for women than men and for younger than

TABLE 3

Mortality among trial participants, 1985-1991

Cause of death	General population trial		Dysplasia trial	
	[n]	Percentage	[n]	Percentage
		%		%
Cancer	[792]	37	[176]	54
Esophagus	[360]	17	[82]	25
Stomach	[331]	16	[77]	24
Lung	[31]	1	[2]	1
Other	[70]	3	[15]	5
Cerebrovascular	[523]	25	[57]	18
Other	[812]	38	[91]	28
Total	[2127]	100	[324]	100

TABLE 4

Relative risks of cause-specific mortality by sex and age according to vitamin and mineral supplement in the general population trial<sup>1</sup>

Cause of death	Males				Females				Age <55 y <sup>2</sup>				Age ≥55 y <sup>2</sup>			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Cancer	1.07	0.94	0.95	0.93	0.86	1.04	1.22	0.79 <sup>3</sup>	0.84	0.98	0.85	0.71 <sup>4</sup>	1.03	0.99	1.16	0.94
Cerebrovascular	1.04	0.87	1.06	0.86	0.94	0.99	1.00	0.96	1.15	0.96	0.89	0.80	0.96	0.92	1.08	0.94
Other	1.00	1.03	0.85	0.97	1.12	0.93	1.12	0.95	1.00	1.11	0.97	1.16	1.06	0.95	0.94	0.90
Total	1.03	0.96	0.93	0.93	0.97	0.99	1.12	0.89	0.96	1.02	0.90	0.87 <sup>3</sup>	1.02	0.96	1.05	0.93

<sup>1</sup> Supplements as follows: A, retinol and zinc; B, riboflavin and niacin; C, vitamin C and molybdenum; and D, β-carotene, vitamin E, and selenium.<sup>2</sup> Age at start of trial.<sup>3,4</sup> Significantly different from those not receiving supplement D: <sup>3</sup>  $P < 0.05$ , <sup>4</sup>  $P < 0.01$ .

older individuals. These differences are consistent with a greater protection for groups at lower risk in this population (although still at high risk by world standards), who perhaps are somewhat more amenable to a protective effect of nutritional supplementation. The sex and age differences were not significant, however, and whether they are etiologically meaningful is not clear. For total mortality, the effects of the β-carotene, vitamin E, and selenium supplements were similar in men and women and only somewhat more pronounced at younger ages.

The most notable difference between men and women occurred in the dysplasia trial. A significant 55% reduction in cerebrovascular disease mortality occurred among men who received the multiple vitamin and mineral supplements, but only a 10% decrease was seen among women. Although unanticipated before the start of the trial, this large difference may reflect a genuinely greater benefit for men, who had about a 40% higher risk of cerebrovascular disease mortality than did women in this population. On the basis of blood pressure readings taken in participants surviving the intervention period, a significantly reduced prevalence of hypertension also was found in those receiving the multiple vitamin and mineral

supplements (7). Again, this beneficial effect was found only in men, suggesting a mechanism for the sex difference in cerebrovascular disease mortality. Reasons for a greater influence of the vitamins and minerals on both hypertension and stroke in men than in women are not known.

Additional follow-up of the two trials' participants is ongoing even though the interventions ceased in 1991. These ongoing studies should continue to provide useful information about the long-term effectiveness of supplementation with specific nutrients in this population, who has limited dietary options and an exceptionally high risk of cancer. □

TABLE 5

Relative risks of cause-specific mortality by sex and age among those receiving vitamin and mineral supplementation in the dysplasia trial

Cause of death	Males	Females	Age <55 y	Age ≥55 y
Cancer	0.97	0.92	0.81	1.02
Cerebrovascular	0.45 <sup>1</sup>	0.90	0.82	0.59
Other	1.09	1.12	1.48	0.99
Total	0.90	0.96	0.95	0.91

<sup>1</sup> Significantly lower than rate among those receiving placebo,  $P < 0.05$ .

## REFERENCES

- Li JY. Epidemiology of esophageal cancer in China. *Monogr Natl Cancer Inst* 1982;62:113-20.
- Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. I. Epidemiology. *Cancer Causes Control* 1991;2:325-57.
- Li B, Taylor PR, Li JY, et al. Linxian nutrition intervention trials: design, methods, participant characteristics, and compliance. *Ann Epidemiol* 1993;3:577-85.
- Blot WJ, Li JY, Taylor PR, et al. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. *J Natl Cancer Inst* 1993;85:1483-92.
- Li JY, Taylor PR, Li B, et al. Nutrition intervention trials in Linxian, China. Multiple vitamin/mineral supplementation, cancer incidence, and disease-specific mortality among adults with esophageal dysplasia. *J Natl Cancer Inst* 1993;85:1492-8.
- Breslow NE, Day NE. Statistical methods in cancer research. II. The design and analysis of cohort studies. *IARC Sci Publ* 1987;82:1-406.
- Mark SD, Wang W, Fraumeni JF, et al. Lowered risks of hypertension and cerebrovascular disease following vitamin/mineral supplementation: results from the Linxian intervention trials. *Am J Epidemiol* (in press).