

## THE EFFECT OF VITAMIN AND MINERAL SUPPLEMENTATION ON ESOPHAGEAL CYTOLOGY: RESULTS FROM THE LINXIAN DYSPLASIA TRIAL

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The population of Linxian in China has one of the world's highest rates for esophageal/gastric cardia cancer, as well as documented nutritional deficiencies. To determine whether dietary supplementation with a multi-vitamin multi-mineral preparation could reduce the risk of esophageal cancer and favorably influence precursor lesions, 3,318 individuals age 40-69 with cytologically determined grade 1 or grade 2 esophageal dysplasia were randomly assigned to receive either an active multi-vitamin multi-mineral supplement or a placebo. Pills were distributed at monthly visits and incident cancers or deaths were recorded. At 30 and 72 months subsequent to randomization all living participants without a known incident cancer were asked to undergo repeat cytological examination of their esophagus. Based on these procedures participants were classified as having no dysplasia, dysplasia grade 1, dysplasia grade 2 or near cancer dysplasia. Diagnoses of cancer were based on the cytology findings plus available histologic, radiologic and clinical materials. At the end of the study there was little overall difference in cumulative risk of esophageal cancer between those receiving vitamin/mineral supplementation and those receiving placebo. There was, however, a significant increase in reversion to non-dysplastic cytology among the group receiving the active treatment. The odds of not having any dysplasia at the two post-randomization screens was 1.23 times higher in the active treatment group than in the placebo group. Within each treatment group higher categories of dysplasia were associated with higher rates of cancer.

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Some of the world's highest incidence of esophageal cancer occurs in parts of north central China; in the country of Linxian as many as 1 in 3 persons die of this neoplasm (Li, 1982). This excess risk is particularly pronounced among persons with esophageal dysplasia, a spectrum of precancerous lesions affecting over 20% of adults in this rural county (Shen *et al.*, 1993). In 1984 3,318 individuals from Linxian with grade 1 or grade 2 dysplasia were randomized to one of two treatment arms: the treated group (RX group) received a multi-vitamin and multi-mineral supplement; the placebo group (PL group) received an inert placebo. In an earlier report on the Linxian Dysplasia Trial (Li *et al.*, 1993a) we showed that at the end of the study period there were no significant differences between the RX group and the PL group with regard to cumulative cancer incidence. We now use results from cytologic examinations on the severity of esophageal dysplasia, offered almost all participants at both 30 and 72 months subsequent to commencement of the intervention, to assess whether treatment had an impact on pre-malignant disease and whether the effect of treatment was constant over time or instead only appeared after a latency period. As a related question, we examine whether the cytologically diagnosed dysplasias, which we shall call intermediate states, are themselves prognostic for cancer or death.

### METHODS

#### Design

Details of the design and execution of the Dysplasia Trial have been described (Li *et al.*, 1993b; Dawsey *et al.*, 1994). In

brief, 3,318 men and women between the ages of 40 and 69 with cytologic evidence of grade 1 or grade 2 dysplasia began receiving daily supplementation with multiple vitamins and minerals (1,657) or placebo (1,661) in May 1985 (time T1). The supplements included a total of 26 vitamins and minerals at doses typically 2-3-fold higher than the U.S. Recommended Daily Allowances. Distribution of the pills continued through April 1991. The subjects were recruited from 3 communes in Linxian, mostly from persons whose dysplasia was diagnosed during a mass balloon swallow cytology examination in late 1983. Treatment was randomly assigned within blocks of 10 defined by commune, sex and age. Good balance was achieved on all measured risk factors except for initial grade of dysplasia (Li *et al.*, 1993b): the RX group had a higher proportion of grade 2 dysplasias ( $p = .02$ ).

Mortality and cancer incidence through the intervention period were ascertained by essentially complete surveillance of the participants, and cause of death and type of cancer were determined by a review of pathology and cytology slides, X-rays, and clinical records by a committee of senior Chinese and American diagnosticians (Li *et al.*, 1993b). At approximately 30 months (October-December 1987, time T2) and 72 months (March-May 1991, time T3) subsequent to the commencement of active treatment, all living participants without known incident cancer were asked to undergo esophageal balloon cytology sampling. About one-quarter of the participants were evaluated further by endoscopy at time T2, and about one-eighth were endoscoped at time T3. The cytologic smears were interpreted by Chinese cytologists without knowledge of treatment group, using previously published Chinese cytologic criteria (Shen *et al.*, 1993; Shu, 1983). At both T2 and T3, all participants were classified into 1 of 5 outcome categories: (1) no dysplasia (including the cytologic categories of normal and hyperplasia), (2) grade 1 dysplasia, (3) grade 2 dysplasia, (4) near cancer dysplasia or (5) cancer. The first 4 outcome categories were based on the cytologic diagnoses from the balloon screening examinations; the category of cancer was based on any cytologic, histologic, radiologic or clinical diagnosis confirmed by the committee of senior Chinese and American diagnosticians. Individuals whose samples had both squamous and columnar cells on their cytology smears were placed into the category of their most severe lesion. Thus in this analysis we make no distinction between squamous dysplasia (likely to be arising from the esophagus) and glandular dysplasia (likely to be arising in the gastric cardia) and refer to both as esophageal dysplasia. Similarly, for the purposes of this analysis the term "esophageal cancer" includes cancers of the esophagus and stomach (consisting mostly of gastric cardia). Cardia cancers also occur in excess in this region, and any analysis which separates esophageal and stomach cancers requires assumptions about their joint distribution. A more detailed breakdown by cell type and location of

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the esophageal and gastric cancers diagnosed during the Dysplasia Trial can be found in Li *et al.* (1993b).

#### Statistical analysis

We call each of the 5 mutually exclusive outcome categories into which an individual may be classified at T2 and T3 as "J states" and indicate which category an individual is in at a given time by the notation  $J = j$  where  $J = 0$  indicates no dysplasia;  $J = 1$ , grade 1 dysplasia;  $J = 2$ , grade 2 dysplasia;  $J = 3$ , near cancer dysplasia; and  $J = 4$  cancer. By the end of the study each individual can be characterized as having followed 1 of 21 outcome histories; for example, a person might be grade 1 dysplasia at T2 ( $J = 1$ ) and near cancer at T3 ( $J = 3$ ). There are 21 rather than 25 such histories since an individual who is  $J = 4$  (has esophageal cancer) at T2 is also  $J = 4$  at T3. If we had full information on all participants at T2 and T3, we could estimate the probabilities of each of these outcome histories by their observed frequencies, and, applying a method for analyzing longitudinal categorical data proposed by Koch *et al.* (1977), we could obtain unbiased estimates of the desired treatment comparisons. In this study 2 factors prevent our having full information on all participants: not all participants agreed to be re-instrumented at T2 and T3 (Dawsey *et al.*, 1994); some individuals died from causes other than esophageal cancer (Li *et al.*, 1993a) and thus could not be reassessed. In all, 34% of people were missing outcome information at either T2 (7%), T3 (18%) or both times (9%). To estimate the probabilities of the outcome histories with such missing data we used an adaptation of the Koch approach proposed by Mark and Gail (1994). In contrast to the common approaches which require the outcomes to be missing completely at random (Little and Rubin, 1987), the approach of Mark and Gail (1994) relaxes this assumption to allow, for example, an individual's chance of participating at T3 to depend upon what he/she was doing at his/her dysplastic status at T2.

To control for the potential confounding of treatment effect induced by the unequal distribution of initial grade, most of the analyses will be based on estimates within the 4 categories defined by initial dysplasia grade as well as by treatment (Table I). These categories will be subsequently referred to by the abbreviations RX.1, PL.1, RX.2, PL.2: the first 2 letters indicate whether vitamin and mineral supplementation (RX) or placebo (PL) was assigned, and the number indicates grade 1 or grade 2 dysplasia at the start of the study. In accordance with the matched design of the study, estimates from data in each of these 4 categories are themselves formed from the weighted averages of estimates within the 3 age categories (50 years or younger, 50-60, and older than 60). Due to the large number of categories that would be required to simultaneously account for the matching on sex and commune, we ignore this element of the design. Ignoring these additional matching factors induces no bias in our point estimates. It may, however, make the variance estimates too conservative (*i.e.*, too large). We suspect this increase in variance is negligible, since controlling for these matching factors once at a time vs. not controlling for them at all results in slight changes in the variance estimates that do not alter the substantive nature or strength of the inference. In order to obtain overall estimates of treatment effect, category-specific estimates are combined by the method of weighted least squares.

Regression estimates for the effect of treatment on the logit of the cumulative distribution at T2 and T3 are made using a weighted least squares fit of the empirical cumulative logits in the 4 treatment by initial grade categories (Koch *et al.*, 1977; Mark and Gail, 1994). We obtained smoothed estimates of treatment effect by eliminating from the saturated model (32 parameters) the 3-way interaction terms among time, initial grade of dysplasia and treatment ( $p = 0.48$ ) and the 2-way interaction between initial grade of dysplasia and treatment ( $p = .14$ ). The predicted cumulative distributions obtained

from these regressions were checked to ensure that they increased monotonically with increasing outcome category. The estimated probabilities of transition from states at T2 to states at T3 were obtained by collapsing over initial grade, since given J state at T2, the transitional probabilities were similar in both initial grade strata.

All  $p$  values given are 2-sided. Estimates are given with the 95% confidence intervals in parentheses. The data analysis was performed with the analyst unaware of which group received the vitamin-mineral supplementation and which received the placebo.

## RESULTS

Table I lists the number of individuals in each of the 4 categories defined by treatment and initial dysplasia grade, the number of observed esophageal cancers by T2 and T3 and estimates of the cumulative risk of developing cancer at each of the 2 times. (Note that since the estimates are adjusted for age and missing observations, they cannot be obtained directly by dividing the number of observed cancers by the corresponding number of subjects.) None of the differences in cumulative risk of cancer between the comparable strata in the RX and PL groups are statistically significant at either T2 or T3. Pooling over initial dysplasia grade, we estimate the overall cumulative risk difference (the probability  $J = 4$  in the RX group minus the probability  $J = 4$  in the PL group) at T3 and its 95% confidence interval to be 0.002 (-0.025, 0.029). The relative risks (treatment group to placebo group) of developing cancer within a given interval are 1.00 (0.78, 1.23) for the T1-T2 interval and 0.95 (0.68, 1.22) for the T2-T3 interval. These interval-specific relative risks do not differ significantly from each other; both include the null value of 1 in their 95% confidence intervals.

We could judge the effect of treatment on the full range of J states by comparing the probabilities of each of the 5 different J states in each treatment group at both times. However, to utilize the ordinal nature of the outcomes, it is preferable to compare the cumulative probabilities of being less than or equal to state J, rather than the individual probabilities of being state J. For example, if the cumulative probability of being less than or equal to J state 3 at T3 is higher in one group than the other, then the chance of not developing cancer by T3 is higher in that group. In contrast, the fact that the probability of being in state  $J = 3$  is less in one group than the other does not imply that the overall risk of cancer is less.

Table II contains estimates of the probability of being less than or equal to outcome J at time T2 (rows 1-4) and time T3 (rows 5-8). For example, from the first row of the column labeled " $J \leq 2$ " we see that 83% of the people in the treated group who were initial grade 1 (RX.1) have cytologic outcomes less than or equal to grade 2 dysplasia at time T2. We can also calculate the probability in category RX.1 that a person has dysplasia grade 2 ( $J = 2$ ) at T2 as  $\Pr(J = 2) = \Pr(J \leq 2) - \Pr(J \leq 1) = 0.83 - 0.68 = 0.15$ .

TABLE I - NUMBER OF SUBJECTS AND CANCERS AND ESTIMATED CUMULATIVE RISK OF ESOPHAGEAL CANCER BY TIME 2 (T2) AND BY THE END OF STUDY (T3) FOR CATEGORIES DEFINED BY TREATMENT GROUP AND INITIAL DYSPLASIA GRADE

Number in category	RX.1 1243	PL.1 1302	RX.2 414	PL.2 359
Cancers by T2				
Number observed	79	67	53	49
Cumulative %	0.08	0.07	0.14	0.16
Cancers by T3				
Number observed	141	141	78	68
Cumulative %	0.15	0.14	0.23	0.23

TABLE II - OUTCOMES AT T2 AND T3 BY TREATMENT AND INITIAL DYSPLASIA GRADE

Category	Probability <sup>1</sup> of state J <sup>2</sup> or less			
	J = 0	J ≤ 1	J ≤ 2	J ≤ 3
Time 2				
RX_1	0.33 (.014)	0.68 (.014)	0.83 (.011)	0.92 (.008)
PL_1	0.30 (.014)	0.69 (.014)	0.85 (.011)	0.93 (.007)
RX_2	0.29 (.023)	0.58 (.025)	0.77 (.021)	0.86 (.017)
PL_2	0.20 (.022)	0.52 (.027)	0.74 (.023)	0.84 (.019)
Time 3				
RX_1	0.46 (.016)	0.69 (.015)	0.81 (.012)	0.85 (.011)
PL_1	0.41 (.016)	0.68 (.015)	0.81 (.012)	0.86 (.011)
RX_2	0.39 (.026)	0.61 (.026)	0.72 (.024)	0.77 (.022)
PL_2	0.34 (.028)	0.55 (.029)	0.68 (.027)	0.77 (.023)

<sup>1</sup>Standard errors in parentheses.—<sup>2</sup>J = 0, no dysplasia; = 1, grade 1 dysplasia; = 2, grade 2 dysplasia; = 3, near cancer; = 4, cancer.

TABLE III - TIME-SPECIFIC AND COMMON ODDS RATIOS<sup>1,2,3</sup> FOR THE EFFECT OF TREATMENT ON OUTCOME

	J = 0	J ≤ 1	J ≤ 2	J ≤ 3
Time 2	1.26 (1.06-1.46)	1.06 (0.90-1.22)	0.93 (0.76-1.11)	0.95 (0.72-1.18)
	<i>p</i> = .005	<i>p</i> = .46	<i>p</i> = .48	<i>p</i> = .68
Time 3	1.21 (1.02-1.40)	1.10 (0.93-1.28)	1.05 (0.86-1.24)	0.98 (0.78-1.17)
	<i>p</i> = .020	<i>p</i> = .23	<i>p</i> = .62	<i>p</i> = .84
Common	1.23 (1.08-1.37)	1.08 (0.95-1.21)	0.99 (0.84-1.14)	0.95 (0.77-1.13)
	<i>p</i> < .001	<i>p</i> = .21	<i>p</i> = .93	<i>p</i> = .58

<sup>1</sup>Odds ratios are (ODDS J ≤ j |RX)/(ODDS J ≤ j |PL).—<sup>2</sup>95% confidence intervals in parentheses; *p*-values beneath estimates.—<sup>3</sup>See Table II for definition of J states.

With regard to ascertaining treatment effects, the comparisons of interest in Table II are between the RX and PL groups within the same initial grade stratum; that is, we compare adjacent rows whose categories end in the same number. The most prominent difference is the RX group's greater probability of being non-dysplastic (J ≤ 0) at both initial grade levels and at both times. For those who started with dysplasia grade 2, the probabilities of being grade 1 or better (J ≤ 1) and grade 2 or better (J ≤ 2) are also higher in the treated group at both T2 and T3. In general, the differences between the RX and PL group tend to decrease as J state increases; that is, the tendency for the vitamin-treated group to be shifted toward the lower categories decreases as the severity of the lesion increases. The cumulative differences of cells are slight for J ≤ 3 for both initial grades of dysplasia and for both times, a fact we previously noted when finding no evidence of an effect of treatment on the overall or interval-specific development of cancer.

To provide more formal estimates of the effect of treatment on these cumulative probabilities and to test whether the treatment effect varies with time (T2 vs. T3) or whether the observed differences of the treatment effect across initial dysplasia grade are consistent with chance variation, we fit logistic regression models for ordinal outcomes. The treatment odds ratios (Table III) are adjusted for differences in the prevalence of initial dysplasia grade between the 2 groups. The time-specific odds ratios (rows 1 and 2) can be regarded as the common odds ratio at a given time based on an analysis stratified on initial dysplasia grade (initial dysplasia grade was not a significant effect modifier of treatment, *p* = 0.14). Because there were no significant differences in the effect of treatment on outcome at T2 and T3 (*p* = 0.79), we combine the 2 time-specific odds ratios to obtain the overall common odds ratios (row 3).

The estimate of a common odds ratio (row 3) of 1.23 for level J ≤ 0 (column 1) indicates that the odds of having no dysplasia (J ≤ 0) vs. the odds of having one of the dysplastic states or cancer (J > 0) are 1.23 times higher in the RX group than in the PL group. The confidence interval for this odds

ratio excludes the null value of 1 and the *p* value is highly significant. The estimate of 1.08 in column 2 of the summary odds ratio indicates that the odds of having dysplasia grade 1 or no dysplasia (J ≤ 1) are 1.08 times as great in the RX as in the PL group. Neither this odds ratio nor the other 2 odds ratios (columns 3 and 4) are significantly different from 1. In accord with the observation made in Table II that the treatment differences diminish as J state increases, we see a monotonic decrease in the point estimates as we move across the row.

In Table II we listed the cumulative marginal probabilities at T2 and T3. These marginal probabilities afford a parsimonious description of the overall effect of treatment on outcome. Another aspect of the data is revealed by the cumulative transitional probabilities which describe the probability of being in state J ≤ j at the end of an interval given one's state at the beginning of the interval (Table IV). For example, in row 1, column 1 (J ≤ 0), we see that 60% of persons in the RX group who had no dysplasia (J = 0) at T2 had no dysplasia at T3. In contrast (row 4, column 1), only 32% of individuals in the RX group who had near cancer (J = 3) at time 2 had no dysplasia at time 3. Since in Table II the listed results were stratified on initial grade, the transitional probabilities for the T1-T2 period are identical to the marginal probabilities (top half of Table II) and are not repeated in Table IV.

The transitional analysis provides further insight into the nature of the treatment effect. Comparing the probabilities in the top half of Table IV (the RX group) with the corresponding probabilities in the bottom half (the PL group) we find that the probability of having no dysplasia at the end of an interval is greater in the treated group than in the placebo group for every starting state. The marginal analysis revealed that overall the odds of not having any dysplasia were greater for individuals in the treated groups than for those in the placebo group. The transitional analysis shows that the effect of treatment is to increase the movement into the non-dysplastic state from all 3 of the intermediate dysplastic states.

For each of the interior cells of Table IV, the probability in each cell is greater than the probability in the cell beneath it,

TABLE IV - CUMULATIVE TRANSITIONAL PROBABILITIES IN THE T2 TO T3 INTERVAL

	Probability of J <sup>1</sup> or less at time 3			
	J = 0	J ≤ 1	J ≤ 2	J ≤ 3
J state at T2-treated group				
J = 0	.60 (.026)	.81 (.021)	.90 (.016)	.94 (.013)
J = 1	.49 (.026)	.78 (.021)	.89 (.016)	.93 (.013)
J = 2	.38 (.036)	.69 (.035)	.87 (.025)	.93 (.020)
J = 3	.32 (.048)	.55 (.050)	.75 (.044)	.83 (.038)
J state at T2-placebo group				
J = 0	.52 (.028)	.81 (.022)	.91 (.016)	.94 (.014)
J = 1	.45 (.025)	.74 (.022)	.88 (.017)	.92 (.014)
J = 2	.28 (.034)	.63 (.034)	.81 (.028)	.91 (.021)
J = 3	.23 (.043)	.58 (.052)	.75 (.045)	.86 (.037)

<sup>1</sup>See Table II for definition of J states.

indicating that groups that start an interval one J state lower than another tend to have lower J states at the end of the interval.

We also examined whether dysplasia itself was a significant prognostic factor for cancer. Though Table I shows that individuals with initial grade 2 have higher cumulative risks of cancer than those with initial grade 1 in both the RX and PL groups, this finding may have resulted from the fact that endoscopic assessment at T2 was preferentially offered to those whose initial dysplasia was grade 2. However, the sample of individuals chosen for endoscopy at T3 did not depend on prior dysplastic state (Dawsey *et al.*, 1994). Table V contains estimates of the cancer rates in the final 42 months of the study (T2-T3) for each level of dysplasia at 30 months (T2). By observing within each treatment group how these rates change with J state, we can judge whether these intermediate states have predictive significance for cancer. Though the magnitude of many of these individual differences are small compared to their standard errors, overall the rates do increase monotonically with increasing J state. A non-parametric test (Hollander and Wolfe, 1973) for increasing cancer rate with increasing grade of dysplasia is significant ( $p = .002$ ).

#### DISCUSSION

We found no evidence for a beneficial effect of the vitamin-mineral supplementation treatment on the chance of developing esophageal and stomach cancers in the 6-year period of this study. This agrees with our earlier report (Li *et al.*, 1993a) in which no significant differences in overall mortality, overall cancer incidence or esophageal and stomach cancer incidence were found. We have, however, demonstrated an effect of the treatment on dysplasia: persons in the treated group were 1.2 times as likely to have no dysplasia on follow-up exam at years 2.5 and 6 as were persons in the placebo group. The point estimates of the effect on the other intermediate grades decreased in magnitude with increasing severity and were not statistically significant.

One question that naturally arises is whether the intermediate dysplasias are themselves important predictors of subsequent cancer risk, and if so, would the increased prevalence of lower grades in the treated group at T2 eventually translate into lower rates of cancer and death. Within this study the best evidence for the prognostic significance of these intermediate lesions comes from comparing the rates at which persons in the 4 non-cancerous J states at time 2 developed cancer over the ensuing 42 months. We found that persons with more advanced lesions at time 2 develop cancer at higher rates (Table V). There are several ways of addressing the question of whether a longer follow-up would reveal a benefit of the vitamin intervention on cancer incidence and mortality. If one extrapolated present results for 7 years by assuming that an individual's subsequent risk is only dependent upon his current

TABLE V - RATES<sup>1</sup> OF CANCER IN THE T2-T3 INTERVAL GIVEN J STATE AT START OF INTERVAL

J <sup>2</sup> State at T2	RX group	PL group
J = 0	16.7 (3.5)	16.0 (3.7)
J = 1	17.5 (3.6)	19.5 (3.7)
J = 2	18.8 (5.3)	24.9 (5.9)
J = 3	47.2 (5.9)	37.8 (6.2)

<sup>1</sup>Rates are per 1,000 person years and are estimated by assuming a constant hazard of cancer in the 42-month interval. Standard deviation is shown in parentheses. The test statistic for trend is the sum of Jonckheere's (Hollander and Wolf, 1973) distribution free test statistic within each treatment group. The two-sided test for trend obtained from the exact permutation distribution yielded  $p = 0.002$ .—<sup>2</sup>See Table II for definition of J states.

J state (a first order Markov assumption) and that the transition rates observed between T2 and T3 will persist, one projects negligible treatment differences with continued follow-up.

However, data from an observational study on esophageal cancer in Linxian (Dawsey *et al.*, 1994) casts doubts on these assumptions. In that study, follow-up was obtained on approximately 12,000 persons in Linxian who had undergone esophageal balloon cytology 15 years earlier. Using those data and the non-dysplastic designation as defined in our analysis as the baseline category, we calculated the 15-year relative risk of esophageal or gastric cardia cancer for the individuals who were initially dysplasia grade 1, dysplasia grade 2 and near cancer (called suspicious for cancer at the time of that screen) as 1.5, 1.8 and 4.6 respectively. In contrast, Markov projections of the cancer rates for the placebo group in the present trial yielded relative risks of 1.04, 1.10 and 1.22 at 14 years. This discrepancy suggests that the simple projections made under the stationary, first-order Markov model underestimate the long-term prognostic significance of differences in grade and, hence, may underestimate the long-term benefit of treatment.

We are left with the observation that treatment has a beneficial effect on dysplasia and that dysplasia is a risk factor for cancer but that treatment as yet has no effect on the development of esophageal cancer within 6 years. There are several ways of reconciling these observations. Those with dysplasia may constitute a mixed population, some of whom are at increased risk for progression to cancer and others who are not. The vitamin-mineral supplementation may only move the latter toward normality and have no effect on the former. Alternatively we may be observing a benefit of treatment only on the early lesions of a progressive disease, because the intervention occurred too late for those who were destined to develop cancer in this 6-year interval. For instance, suppose that increasing states of dysplasia correspond to progressive advance along a pathway that leads to transformation into a

cancer cell. The vitamin-mineral supplementation affects the grade of the lesions and thus reduces the progression toward cancer. However, once the cell has been transformed, treatment does not affect the rate of progression to detectable cancer. If most of the cancers detected during this 6-year interval arose from cells already transformed at the start of the study, we might well find no effect of treatment on cancer rates even if the treatment-reduced severity of dysplasia will eventually be accompanied by a reduction in long-term risk of cancer. We might, however, find an effect on the intermediate grades and additionally that the intermediate grades are prognostic for esophageal cancer if, as is likely, the chance of having an already extant transformed cell increases as grade increases.

To perform any analysis of these data requires making assumptions regarding the J states of those 2 sets of persons on whom we were missing measurements: individuals known to have died from other causes and individuals who, though alive, elected not to participate in the outcome assessment procedures at T2 and/or T3. When we include the former by broadening the final category ( $J = 4$ ) to include all cancers or death, the conclusions of the analysis are qualitatively unchanged. For the other individuals, our methods might be biased if the decision to participate in the measurement

procedure depended not only on past measured J states but also on current unmeasured J states. Since the intermediate lesions as well as the vast majority of the detected esophageal cancers were asymptomatic and hence unknown to the study individuals, we feel that bias from such differential participation rates is unlikely.

The inability to detect a treatment effect on cancer incidence cannot be ascribed to lack of power or non-compliance to treatment. The experiment had a power of over 95% to detect an overall difference in cumulative incidence of esophageal and stomach cancer of 4% based on a 2-sided  $\alpha = 0.05$  level test. Judging from pill disappearance and biochemical data (Li *et al.*, 1993b), compliance with the assigned treatment regime was excellent. Thus the data present strong evidence that the vitamin-mineral intervention is not an effective preventative for cancer or death within a 6-year period for persons with dysplasia. Treatment does appear to be effective in shifting dysplastic lesions of all severity toward a non-dysplastic state. Without a longer period of study one cannot determine whether this treatment effect will have clinical or public health significance. Although pill distribution ceased in 1991, follow-up of the treated and placebo groups is continuing.

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