What have we learned from the Nutrition Intervention Trials on Cancers in Linxian, China?

The Annual Scientific Meeting of the Korean Society for Preventive Medicine
Seoul, Korea, October 28th~29th, 2004

Youlin Qiao, Philip Taylor et al
Cancer Institute, Chinese Academy of Medical Sciences
National Cancer Institute/NIH, USA
Outline of talk

1. Linxian Nutrition Intervention Trials
   1. Trials
   2. Nested case-cohort studies
   3. 15 year follow-up
   4. Other studies: the chemo-regression study

2. What have we learned
Linxian Nutrition Intervention Trials

- The trials
Etiologic Clues:
Esophageal Cancer in Northern China

- **Carcinogen exposures** - Pickled vegetables, moldy foods, nitrosamines, mycotoxins
- **Physical irritation** - Silica, hot food
- **Genetics** - Family Ca history, L-myc gene
- **Smoking & alcohol drinking??**
- **Nutritional inadequacies** - Riboflavin, vitamins A & C, Zn, Se, folate
Linxian Nutrition Intervention Trials

<table>
<thead>
<tr>
<th>Dysplasia Trial</th>
<th>General Population Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Population:</strong></td>
<td>3,318 adults 40-69yr, cytologic dysplasia</td>
</tr>
<tr>
<td><strong>Design:</strong></td>
<td>2-arm, double-blind, placebo-controlled</td>
</tr>
<tr>
<td><strong>Intervention:</strong></td>
<td>26 vitamins, minerals</td>
</tr>
<tr>
<td><strong>Duration:</strong></td>
<td>6 yr</td>
</tr>
<tr>
<td><strong>Primary endpoint:</strong></td>
<td>Esophageal cancer incidence, mortality</td>
</tr>
</tbody>
</table>

Li et al, Ann Epidemiol 1993
## General Population Trial – Linxian
### Treatment factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>Micronutrients</th>
<th>Dose/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Retinol</td>
<td>5000 IU</td>
</tr>
<tr>
<td></td>
<td>Zinc</td>
<td>22.5 mg</td>
</tr>
<tr>
<td>B</td>
<td>Riboflavin</td>
<td>3.2 mg</td>
</tr>
<tr>
<td></td>
<td>Niacin</td>
<td>40 mg</td>
</tr>
<tr>
<td>C</td>
<td>Ascorbic acid</td>
<td>120 mg</td>
</tr>
<tr>
<td></td>
<td>Molybdenum</td>
<td>30 μg</td>
</tr>
<tr>
<td>D</td>
<td>Beta-carotene</td>
<td>15 mg</td>
</tr>
<tr>
<td></td>
<td>Vitamin E</td>
<td>30 mg</td>
</tr>
<tr>
<td></td>
<td>Selenium</td>
<td>50 μg</td>
</tr>
</tbody>
</table>
General Population Trial – Linxian

Summary of trial results

For beta-carotene, vitamin E, and selenium (factor D), significant reductions in mortality for:

- Total mortality (9%)
- Total cancer mortality (13%)
- Stomach cancer mortality (21%)

Blot et al, JNCI 1993
General Population Trial
Cumulative Total Deaths (%)
Since Start of Intervention
MARCH 1986 – MAY 1991

Supplementation:
- NO BETACAR/MT E/SE
- BETACAR/MT E/SE

Months of Intervention
General Population Trial
Cumulative Cancer Deaths (%)
Since Start of Intervention
March 1986 - May 1991

Supplementation:
- BETACAR/VIT E/SE
- NO BETACAR/VIT E/SE

Blot et al, JNCI 1993
General Population Trial
Cumulative Stomach Cancer Deaths (%) Since Start of Intervention
March 1986 - May 1991

Supplementation:
- BETACAR/VIT E/SE
- NO BETACAR/VIT E/SE

Months of Intervention
The combination of beta-carotene, vitamin E, and selenium reduced total mortality, total cancer mortality, and stomach cancer mortality.

Initial effects seemed evident as early as 1 to 2 years after the start of the trial, but were most prominent after 3 to 4 years of intervention.
General Population Trial – Linxian
Nested case-cohort studies

**Study description**

- All incident esophageal, cardia, and non-cardia stomach cancers diagnosed during the trial (from 1986-91)
- Random sample of cohort stratified on age and gender
- Baseline (1985, pre-intervention) sera analyzed for selenium, carotenoids, vitamin E

**Analytic group**

- Esophageal cancer = 590
- Cardia cancer ~ 402
- Non-cardia cancer = 87
- Non-case subcohort ~ 1053
- Cox proportional hazards regression
**General Population Trial – Linxian**

**Distribution of analytes from nested case-cohort studies**

<table>
<thead>
<tr>
<th></th>
<th>Selenium</th>
<th>β-carotene</th>
<th>α-tocopherol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Linxian</strong></td>
<td>71</td>
<td>43</td>
<td>804</td>
</tr>
<tr>
<td></td>
<td>(51, 93)</td>
<td>(14, 124)</td>
<td>(577, 1128)</td>
</tr>
<tr>
<td><strong>U.S.</strong></td>
<td>124</td>
<td>147</td>
<td>969</td>
</tr>
<tr>
<td>(NHANES III, 1988-94)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(108, 142)</td>
<td>(64, 351)</td>
<td>(681, 1554)</td>
</tr>
</tbody>
</table>
# General Population Trial – Linxian

## RRs for cancer sites by serum analytes (quartiles)

<table>
<thead>
<tr>
<th>Cancer site</th>
<th>Analyte*</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>P trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esophagus</td>
<td>Se</td>
<td>1.00</td>
<td>0.84</td>
<td>0.66</td>
<td>0.56</td>
<td>$&lt;10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>1.00</td>
<td>1.09</td>
<td>0.89</td>
<td>1.01</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>AT</td>
<td>1.00</td>
<td>0.79</td>
<td>0.66</td>
<td>0.63</td>
<td>$0.008$</td>
</tr>
<tr>
<td>Cardia</td>
<td>Se</td>
<td>1.00</td>
<td>0.75</td>
<td>0.55</td>
<td>0.47</td>
<td>$&lt;10^{-6}$</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>1.00</td>
<td>0.89</td>
<td>0.76</td>
<td>0.95</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>AT</td>
<td>1.00</td>
<td>0.79</td>
<td>0.70</td>
<td>0.84</td>
<td>0.29</td>
</tr>
<tr>
<td>Non-cardia</td>
<td>Se</td>
<td>1.00</td>
<td>1.20</td>
<td>1.08</td>
<td>1.07</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>1.00</td>
<td>1.94</td>
<td>1.32</td>
<td>1.86</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>AT</td>
<td>1.00</td>
<td>1.32</td>
<td>2.21</td>
<td>2.05</td>
<td>$0.03$</td>
</tr>
</tbody>
</table>

*Se=selenium  
BC=beta-carotene  
AT=alpha-tocopherol

Mark et al, JNCI 2000; and unpublished data
Of the components in the combination, selenium and vitamin E are the most likely beneficial agents. There is no apparent effect for beta-carotene.
Linxian Nutrition Intervention Trials

- Up to 15 year followup
## General Population Trial – Linxian
Number of deaths by cause and time period, 1986-2001

<table>
<thead>
<tr>
<th>Cause</th>
<th>1986-91 (trial)</th>
<th>1991-96</th>
<th>1996-01</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>2528</td>
<td>3555</td>
<td>3644</td>
<td>9727 (100)</td>
</tr>
<tr>
<td>Cancer</td>
<td>992</td>
<td>1245</td>
<td>1005</td>
<td>3242 (33)</td>
</tr>
<tr>
<td>esophagus</td>
<td>448</td>
<td>594</td>
<td>473</td>
<td>1515 [47]</td>
</tr>
<tr>
<td>stomach</td>
<td>406</td>
<td>436</td>
<td>357</td>
<td>1199 [37]</td>
</tr>
<tr>
<td>other</td>
<td>138</td>
<td>215</td>
<td>175</td>
<td>528 [16]</td>
</tr>
<tr>
<td>Stroke</td>
<td>643</td>
<td>1037</td>
<td>1304</td>
<td>2984 (31)</td>
</tr>
<tr>
<td>All other</td>
<td>893</td>
<td>1273</td>
<td>1335</td>
<td>3501 (36)</td>
</tr>
</tbody>
</table>
# General Population Trial – Linxian

RRs for factor D (BC+AT+SE) by cause of death and time period

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>0.91</td>
<td>0.99</td>
<td>0.94</td>
<td>0.95</td>
</tr>
<tr>
<td>Cancer</td>
<td>0.91</td>
<td>0.99</td>
<td>0.94</td>
<td>0.95</td>
</tr>
<tr>
<td>esophagus</td>
<td>1.00</td>
<td>1.05</td>
<td>0.95</td>
<td>1.01</td>
</tr>
<tr>
<td>stomach</td>
<td>0.81</td>
<td>0.95</td>
<td>0.91</td>
<td>0.89</td>
</tr>
<tr>
<td>other</td>
<td>0.96</td>
<td>0.93</td>
<td>0.93</td>
<td>0.94</td>
</tr>
<tr>
<td>Stroke</td>
<td>0.90</td>
<td>1.10</td>
<td>0.94</td>
<td>0.98</td>
</tr>
<tr>
<td>All other</td>
<td>0.92</td>
<td>0.90</td>
<td>0.93</td>
<td>0.92</td>
</tr>
</tbody>
</table>
Nutrition Intervention Trial: General Population

CDF

D:Selenium,V-E,Beta Carotene  

Time from Beginning of Trial to Death (Year)

Endpoint: Total Death
P-value of Log-rank test = 0.007
Nutrition Intervention Trial: General Population

D:Selenium, V-E, Beta Carotene

No
Yes

Time from Beginning of Trial to Cancer Death (Year)

Endpoint: Total Cancer Death
P-value of Log-rank test = 0.138
Nutrition Intervention Trial: General Population

Endpoint: Stomach Cancer Death (Cardia/Noncardia)
P-value of Log-rank test = 0.040
Cumulated mortality of all cancers
(age<55)
Cumulated mortality of all cancers (age≥55)
Cumulated mortality of esophageal* cancer (age<55)

![Graph showing cumulative mortality comparison between intervention group and placebo group over time.](image)
Cumulated mortality of esophageal cancer (age >= 55)

- Intervention group
- Placebo group
General Population Trial – Linxian

Conclusions from 15 year followup

For intervention with beta-carotene, vitamin E, and selenium:

- A cumulative benefit was observed overall
- The post-trial patterns suggest continued benefit, even after intervention has ended
- No post-trial harmful effects were detected
Linxian Nutrition Intervention Trials

Other studies: the chemo-regression study
Chemoprevention of esophageal squamous cell carcinoma: Randomized, placebo-controlled trial in a high-risk population

Objective:

To assess the potential chemopreventive effects of celecoxib and selenomethionine among Linxian residents with histologically mild (mD) or moderate (MD) esophageal squamous dysplasia
Esophageal Squamous Dysplasia

- Esophageal cancer risk increases across histologic grades of mild, moderate, and severe dysplasia.

- Dysplasia can be identified at endoscopy as unstained lesions after iodine dye spray.

- Lower grades of dysplasia remain stable for at least 1-2 years.

Dawsey et al, Cancer 1994
Chemoregression study

Design

- Randomized, double-blind, placebo-controlled
- 2 x 2 factorial design, stratified by sex

Agents
- Celecoxib 200 mg twice per day
- Selenomethionine 200µg once per day

<table>
<thead>
<tr>
<th>Placebo</th>
<th>Celecoxib only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenium only</td>
<td>Celecoxib + Selenium</td>
</tr>
</tbody>
</table>
Chemoregression study: Design/results

- Adults from Linxian with no upper GI symptoms and no history of cancer were screened by endoscopy
- Endoscopy was repeated at baseline immediately before the start of the intervention
- 360 were randomized, 238 were in final analytic cohort
- Intervention duration = 10 months
- Endoscopy was repeated at the end of the intervention
- Primary endpoint = change in histologic grade of the esophageal squamous dysplasia from baseline to the end of the intervention
# Chemoregression study

## Change in histology

<table>
<thead>
<tr>
<th></th>
<th>End of intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression</td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
</tr>
<tr>
<td>All subjects (n=238)</td>
<td>90</td>
</tr>
<tr>
<td>Moderate dysplasia (n=123)</td>
<td>55</td>
</tr>
<tr>
<td>Mild dysplasia (n=115)</td>
<td>35</td>
</tr>
</tbody>
</table>
Celecoxib

- Progression:
  - All Subjects: p = 0.78
  - MD at Baseline: p = 0.71

- Stable:
  - Yes: 44, 50, 47
  - No: 46, 47, 38

- Regression:
  - Yes: 36, 40, 44
  - No: 34, 47, 26

Yes = Celecoxib
No = No Celecoxib
Selenomethionine

- Progression:
  - All Subjects: p = 0.08
  - MD at baseline: p = 0.02

- Stable:
  - All Subjects: 47
  - MD at baseline: 42

- Regression:
  - All Subjects: 39
  - MD at baseline: 21

Yes = Selenium
No = No Selenium
Chemoregression study

Summary

- Selenomethionine 200 µg per day for 10 months improved esophageal squamous dysplasia among subjects with mild dysplasia (mD) at baseline.

- Celecoxib 200 mg twice per day for 10 months had no apparent effect on esophageal squamous dysplasia.

- Further study of selenomethionine as a chemopreventive agent in premalignant lesions of the esophagus appears warranted.
Studies in Linxian
Conclusions

- There is strong and consistent evidence for the role of selenium and vitamin E in the prevention of upper gastrointestinal cancer in this population.

- Population supplementation or fortification should be strongly considered as a primary prevention strategy.
What have we learned from NIT and other completed trials?

- Lag-to-effect
- Effective duration
- Efficacious dose
- Factorial designs
- Intermediate endpoints
- Toxicity
Insights from NIT and other completed trials

**Lag-to-effect**

- Lag-to-effect of intervention on cancer (mortality) appears to be longer than our initial assumptions allowed (≈ 2+ years).

- Lag-to-effect on total mortality is complicated (a mixture of effects from different causes with different lags). There is evidence for early benefit (within 2 years) that is clear by 4 years.
Insights from NIT and other completed trials

**Effective duration**

- Lag-to-effect impacts duration of intervention

- The continued separation of survival curves throughout interventions suggests that benefits would be greater if interventions were longer
Insights from NIT and other completed trials

Efficacious doses

- Benefits were observed with modest doses (with exception of beta-carotene in ATBC Study)

- More is not always better (and can be worse!)
Insights from NIT and other completed trials

Factorial designs

- Prevention trials with complicated factorial designs can be successfully implemented.

- Despite multiple comparison problems, we were able to test multiple hypotheses at the same time for essentially the same cost (two for the price of one!)
Insights from NIT and other completed trials

**Intermediate endpoints**

- Some intermediate endpoints appear to be highly predictive of future cancer (e.g., esophageal dysplasia → cancer)

- There appears to be concordance of intermediate endpoints with cancer endpoints regarding intervention findings

- In the future, smaller and shorter interventions with intermediate endpoints may be valid tests of effect
Insights from NIT and other completed trials

Toxicity

- There is potential toxicity even from most apparently benign agents (e.g., beta-carotene)

- We must consider the possibility of toxicity for all agents evaluated
Collaborators

- **CICAMS**
  - Li Bing, Li Jun-Yao, Wang Guo-Qing, Dong Zhi-Wei, Qiao You-Lin (and many, many more!)

- **NCI**
  - William Blot, Sandy Dawsey, Steven Mark

- **Other**
  - CS Yang, Paul Limburg
Thanks for Listening and Welcome for Collaboration!

Mail Address & Telecommunication:

Department of Cancer Epidemiology  
Cancer Institute, CAMS  
P. O. Box 2258,  
Beijing 100021, PR China  
Tel/Fax: 86-10-6771-3648  
E-mail: qiaoy@public.bta.net.cn  
Website: www.cicams.ac.cn/epi