Folate and Neural Tube Defects

James Mills, Statistics and Prevention Research, National Institutes of Health
Early in an embryo's development, a strip of specialized cells called the notochord (A) induces the cells of the ectoderm directly above it to become the primitive nervous system (i.e., neuroepithelium). The neuroepithelium then wrinkles and folds over (B). As the tips of the folds fuse together, a hollow tube (i.e., the neural tube) forms (C) the precursor of the brain and spinal cord. Meanwhile, the ectoderm and endoderm continue to curve around and fuse beneath the embryo to create the body cavity, completing the transformation of the embryo from a flattened disk to a three-dimensional body. Cells originating from the fused tips of the neuroectoderm (i.e., neural crest cells) migrate to various locations throughout the embryo, where they will initiate the development of diverse body structures (D).
Causes of NTDs-Clues from early Epidemiological Studies

- In the UK, lower social class women had much higher rates
- There is often a strong family history
- These observations suggested that environmental and a genetic factors were important
### Maternal Vitamin Levels in NTD Pregnancies

<table>
<thead>
<tr>
<th></th>
<th>NTD</th>
<th>Controls</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RCF (ng/mL)</strong></td>
<td>141</td>
<td>228</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Vitamin C (WBC)</strong></td>
<td>23.9</td>
<td>34.5</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Smithells, et al., 1976
Does folic acid prevent recurrence of NTDs?

MRC Vitamin Study Research Group
Prof. Nicholas Wald
MRC Study Design

- Women with previous affected pregnancy
- Double blind randomized trial
- Folic acid, other vitamins, both, neither
- 1195 informative pregnancies
MRC Trial Results

- Folic acid was highly protective - relative risk 0.28 (CI 0.12-0.71)
- Other vitamins did not show a significant protective effect - relative risk 0.80 (CI 0.32-1.72)
Does folic acid prevent occurrence of NTDs?

Czeizel and Dudas
Czeizel and Dudas Study
Design

- Women attending a preconception clinic
- Randomized blinded trial
- Multivitamins with folic acid, trace elements (Cu, Mn, Zn, Vit. C-7.5mg)
- 4156 informative pregnancies
Czeizel and Dudas Results

- The trace element group had 2.29/1000 NTDs
- The vitamin group had 0/1000
- The difference was significant (p=0.029)
- What element in the multivitamin was responsible?
How does folic acid prevent NTDs?

- Is it a deficiency?
- Is it a problem with absorbing the vitamin?
- Is it a metabolic problem?
Plasma Sample Collection

- 3 major Dublin maternity hospitals (90% of births in area)
- Collected at first prenatal visit (1986 – 1990)
- 56,049 samples obtained (70% of all deliveries)
Plasma Folate

Cases 3.52 ± 3.1 μg/L
Random Controls 4.54 ± 4.3 μg/L
P = 0.004
Plasma B12

Cases    263 ± 103 ng/L
Random Controls  297 ± 111 ng/L

P = 0.008
<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Combined Control Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine</td>
<td>8.62 ± 2.8 μmol/L</td>
<td>7.96 ± 2.5 μmol/L</td>
</tr>
<tr>
<td><em>P</em></td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>
What is the genetic problem?
5,10-Methylenetetrahydrofolate Reductase (MTHFR) Study

- Thermolabile variant—MTHFR 677 C→T
- A → V substitution
- Reduced activity
MTHFR Genotypes in NTD Cases and Controls

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 83</td>
<td>N = 109</td>
</tr>
<tr>
<td>TT</td>
<td>18.3% (15)</td>
<td>6.1% (6)</td>
</tr>
<tr>
<td>CT</td>
<td>39.0% (32)</td>
<td>43.4% (43)</td>
</tr>
<tr>
<td>CC</td>
<td>42.7% (36)</td>
<td>60.5% (60)</td>
</tr>
</tbody>
</table>
Results – NTD Cases

- Thermolabile allele frequency
  - OR = 1.58 (1.01 – 2.46), P = 0.04

- Homozygosity
  - OR = 3.47 (1.28 – 9.41), P = 0.01
Summary

- NTDs are the result of a genetic-environmental interaction
- Availability of folate is the environmental factor
- Abnormal genes for folate enzymes are the genetic factor
Conclusion

- More folate (exogenous folic acid) is needed to overcome the genetic defect
US PHS Recommendations

- All women of childbearing age capable of becoming pregnant should consume 400 μg of folic acid per day
- Care should be taken to keep total folate consumption under 1000 μg per day except under the supervision of a physician
Can dietary changes provide sufficient folate?

- Food folate is less-well absorbed
- Do you want to eat two pounds of spinach every day?
How can folic acid be delivered?

- 400 micrograms above current dietary intake requires supplement use or fortification

- Will people take supplements?
How can folic acid be delivered effectively?

- Would you take folic acid if you were not planning to get pregnant?

- Educational programs have had mixed results—increasing vitamin taking but not reaching all women at risk
Daily Use of FA Among Women 18 – 45, U.S.

March of Dimes, 2004
How can folic acid be delivered effectively?

- Food fortification was introduced because not all women were taking supplements
How much folic acid is necessary?

- Clinical trials used 0.8 and 4.0 mg
- These quantities are impractical for general recommendations
What is the Minimum Effective Dose of Folic Acid for Preventing NTDs?
Minimum Effective Dose:

Why is This a Difficult Question to Answer?
The Definitive Study

Test reduced doses until NTD rates rise

- 400 µg → Protective
- 300 µg → Protective
- 200 µg → Protective
- 100 µg → Not Protective

UNETHICAL
What Do We Know About Effective Dose?

- Clinical trial doses—definitely work, but too high
  - MRC, 1991: 4 mg
  - Czeizel, 1992: 800 µg

- Case control study doses—presumed to work, but are lower doses effective?
  - Most U.S. studies reported on 400 µg
Complication: Genetic Differences in Folate Metabolism

<table>
<thead>
<tr>
<th>MTHFR Genotype</th>
<th>Mean RCF (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>347</td>
</tr>
<tr>
<td>CT</td>
<td>314</td>
</tr>
<tr>
<td>TT</td>
<td>284*</td>
</tr>
</tbody>
</table>

Molloy AM, et al., *Lancet*, 1997; *p<0.01
How Can We Estimate How Much Folic Acid is Needed?
Maternal Red Cell Folate vs. NTD Risk in Dublin

- Study Design
  - Reviewed early pregnancy maternal RCF from nested case-control study of 84 cases NTD and 266 normal controls
  - Logistic regression analysis to examine relationship between RCF and NTD risk

Daly L, et al., JAMA, 1995
Maternal RCF vs. NTD Risk in Dublin

- Continuous dose-response relationship
- RCF < 150 ng/mL Risk → 6.6/1000
- RCF > 400 ng/mL Risk → 0.8/1000

Maternal RCF vs. NTD Risk in Dublin

- Curve continues down?
- Risk → 0.2/1000?
Maternal RCF vs. NTD Risk in Dublin

- Curve flattens out (genetic Mendelian defects)?
- Risk $\rightarrow 0.5/1000$?
- Unable to confirm optimal RCF level, but 400 ng/mL is highly protective
How Much Folic Acid is Needed to Raise RCF to Protective Levels?
NICHD/Trinity College/Health Research Board Trial

- Study design
  - Randomized, placebo-controlled trial
  - 121 patients randomized to receive placebo, 100 µg, 200 µg, or 400 µg folic acid/d
  - Compliance assessed by sign-in sheets over 6 mo. study period

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Initial Median RCF µg/L</th>
<th>Final Median RCF µg/L</th>
<th>Median Change µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>19</td>
<td>335</td>
<td>311</td>
<td>-12</td>
</tr>
<tr>
<td>100 µg/d</td>
<td>22</td>
<td>309</td>
<td>375*</td>
<td>67</td>
</tr>
<tr>
<td>200 µg/d</td>
<td>28</td>
<td>311</td>
<td>475*</td>
<td>130</td>
</tr>
<tr>
<td>400 µg/d</td>
<td>26</td>
<td>350</td>
<td>571*</td>
<td>200</td>
</tr>
</tbody>
</table>

* p < 0.001
RCF and NTD Risk Calculation

- Initial and post-treatment RCF values substituted into regression equation from L. Daly, et al. to derive estimated change in NTD risk

## Results

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>N</th>
<th>Estimated Reduction in NTD Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>100 µg/d</td>
<td>22</td>
<td>22%</td>
</tr>
<tr>
<td>200 µg/d</td>
<td>28</td>
<td>41%</td>
</tr>
<tr>
<td>400 µg/d</td>
<td>26</td>
<td>47%</td>
</tr>
</tbody>
</table>

Minimum estimate
(considering non-compliance):

~200 µg/d will
decrease NTD rate by 41%
How Has Fortification Affected Folate Levels?
Was the FDA estimate correct?

- Mandated fortification was 140 μg of folic acid per 100 g of grain
- Actual levels were often twice the mandated level
Serum Folate Levels
Pre- and Post-Fortification

CDC
- Pre-fortification: 4.8 ug/L
- Post-fortification: 8.2 ug/L
- Increase: 171%

Lawrence
- Pre-fortification: 12.6 ug/L
- Post-fortification: 6.1 ug/L
- Increase: 48%

Jacques
- Pre-fortification: 4.6 ug/L
- Post-fortification: 5.4 ug/L
- Increase: 117%
RCF Levels
Pre- and Post-Fortification

Red Cell Folate (ng/mL)

CDC
- Pre-Fortification: 160
- Post-Fortification: 264
- Increase: 65%

Ray
- Pre-Fortification: 233
- Post-Fortification: 327
- Increase: 40%
Summary

- Fortification probably increases folic acid exposure by 200 µg/day or more

- Serum and red cell folate levels have risen dramatically (by 171% and 65%, respectively)
Actual Experience with Fortification’s Effect on NTDs
The Gold Standard

How much has the additional 200 µg/day decreased NTD rates?
Estimates Vary Because of Ascertainment Problems

<table>
<thead>
<tr>
<th>Method of Detection</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasonography</td>
<td>55%</td>
</tr>
<tr>
<td>MSAFP</td>
<td>25%</td>
</tr>
<tr>
<td>AF AFP</td>
<td>3%</td>
</tr>
<tr>
<td>Delivery</td>
<td>17%</td>
</tr>
</tbody>
</table>

Experience: U.S. data on prevention of NTDs

- Only two studies
- Modest decrease - 19-26%
- Prenatal cases missed or incompletely ascertained
Experience: Nova Scotia

- Population based study of all NTDs from live births, stillbirths, and terminations
- Compared pre- and post-fortification at 150 µg/100 g
- Total NTD incidence fell by 54%
  - 2.58/1000 → 1.17/1000

Persad VL, et al., CMAJ, 2002
Estimated Effect

- Canada: fortification at 150 µg /100 g grain $\rightarrow$ 50% reduction

- U.S. data with comparably ascertained cases are still not widely available
Conclusions
Conclusions

- It is difficult to pinpoint the lowest effective dose of folic acid
- Data from Ireland indicate 200 µg/day may prevent at least 40% of NTDs
Conclusions

- Actual experience in Canada indicates estimated doses of 200+ µg/day will prevent 50%
- In the U.S., ~50% prevention may be the maximum, or ~70% prevention may be possible—have we “maxed out?”
What else is food fortification doing?
How much folic acid is safe?

- The PHS and IOM expressed concerns about masking of B12 deficiency
- Safety of long term high dose exposure in children is not established
Are There Problems With Folic Acid Fortification?

- Masking of $B_{12}$ deficiency
- Increase in multiple gestations
- Worsening of epilepsy
- Blocking of folate antagonist drugs
Masking of $B_{12}$ Deficiency

- $B_{12}$ deficiency anemia can be corrected by folic acid
- Neurologic damage progresses despite folic acid; may be irreversible
Masking of B\textsubscript{12} Deficiency

- Problem in the elderly
  - \( \geq 12\% \) of elderly in Framingham cohort are B12 deficient

- Protean neurologic signs make diagnosis difficult

How great is the risk for masking?

- The only data come from old, anecdotal reports
- Has been seen at doses as low as 400 μg/day
- Does not always occur at very high doses
- Collecting more data would be unethical
Are More $B_{12}$ Deficient Patients Presenting Without Anemia Since Fortification?

- All patients with low $B_{12}$ level were identified from Washington, DC VA laboratory
- Before, during and after implementation of fortification--1992-2000
- Proportion presenting without anemia was calculated

Proportion of Subjects With Low $B_{12}$ Without Anemia

- Pre-fortification (1992-1996): 39.2%
- Optional Fortification (1996-1998): 45.5%
- Post-Fortification (1998-2000): 37.6%

Age-adjusted OR 1.00; 95% CI: 0.88, 1.13; P=0.96
Caveats

- Although neurologic data were available, they were not sufficient to distinguish B\textsubscript{12} related problems from other neurologic disease

- Therefore, we do not know how many subjects without anemia actually had masking
Caveats

- Supports safety of current exposure levels
- Safety of higher fortification levels cannot be inferred
Multiple Gestation

- Increased morbidity and mortality
- Increased health care costs
Does Folic Acid Increase Multiple Gestation Rates?

- Czeizel (Hungary) reported 40% increase in twinning in a clinical trial

## Number and Rate of Multiple Gestations

<table>
<thead>
<tr>
<th></th>
<th>Folic Acid/MV Group</th>
<th>Trace Element Group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Births</strong></td>
<td>89/2243 (3.97%)</td>
<td>58/2199 (2.64%)</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Pregnancies</strong></td>
<td>44/2198 (2.00%)</td>
<td>29/2170 (1.34%)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Folic Acid and Multiple Gestation: Data Summary

Meta-analysis
- UK, 1998
- Sweden, 2001
- California, 2003
- China, 2003
- Texas, 2003

Case - Control
- Atlanta, 1997
- California, 1997

Clinical Trial
- UK, 1991
- Ireland, 1992
- Hungary, 1994
Folic Acid and Epilepsy

- Early case reports and uncontrolled studies suggested folic acid increased seizure severity and frequency
- Subsequent randomized trial data showed no excess risk
Effects on Folate-Antagonist Drugs

Methotrexate (MTX): Widely used:

- ectopic pregnancy
- psoriasis
- non-Hodgkin’s lymphoma
- choriocarcinoma
- leukemia
- osteosarcoma
- rheumatoid arthritis
- inflammatory bowel disease
- mycosis fungoides
- breast/head/neck/ovarian/bladder cancer
Folic Acid and Rheumatoid Arthritis Therapy

- Methotrexate (anti-folate) is a key drug for arthritis
- Folic acid might block its effect
- Studies show no problems
Folic Acid, MTX, and Ectopic Pregnancy

- Prospective study of 20 patients with ectopic pregnancy and baseline plasma folate level
- Pretreatment plasma folate > 20.7 ng/mL associated with failure of single-dose MTX treatment
  - 4/9 (44%) vs. 0/9 (0%), p = 0.02

What other benefits might there be?

- Reduction of folate deficiency anemia-- Proved
Cardiovascular Disease Prevention

- Folate reduces homocysteine
- Homocysteine is associated with CVD risk in case control and prospective studies
- Clinical trials have not shown a preventive effect (84% power to detect a 10% reduction in mortality)
Folate and Cancer

- Folic acid said to promote cancer growth
- High folate diet may prevent cancer, particularly colon cancer (DNA repair?)
- What is the effect on precancerous lesions?
Alzheimer’s Disease

- Conflicting data on benefit of folic acid treatment for prevention
Unknowns

- No studies of long-term, high-dose exposure in children*

- Difficult to monitor for adverse effects
  - No comparison population

*Big eaters
Unknowns

Unexpected adverse effects?

- Some “safe” interventions have unanticipated effects, e.g. DES, O₂
- No comparison population for studies
Summary

- **Masking B\textsubscript{12} Deficiency**
  - One study suggests it is not a problem at current fortification levels
  - Could be at higher levels

- **Multiple Gestation**
  - Many studies now show little increase
Summary

- Blocking anti-folates
  - MTX action may be blocked
Conclusion

Data on safety of folic acid are very limited
Summary

- Food fortification with folic acid is preventing many NTDs
- There have been few studies to look for adverse effects
- The optimal dose of folic acid has not been determined
- There may be other beneficial effects