Folate and Neural Tube Defects

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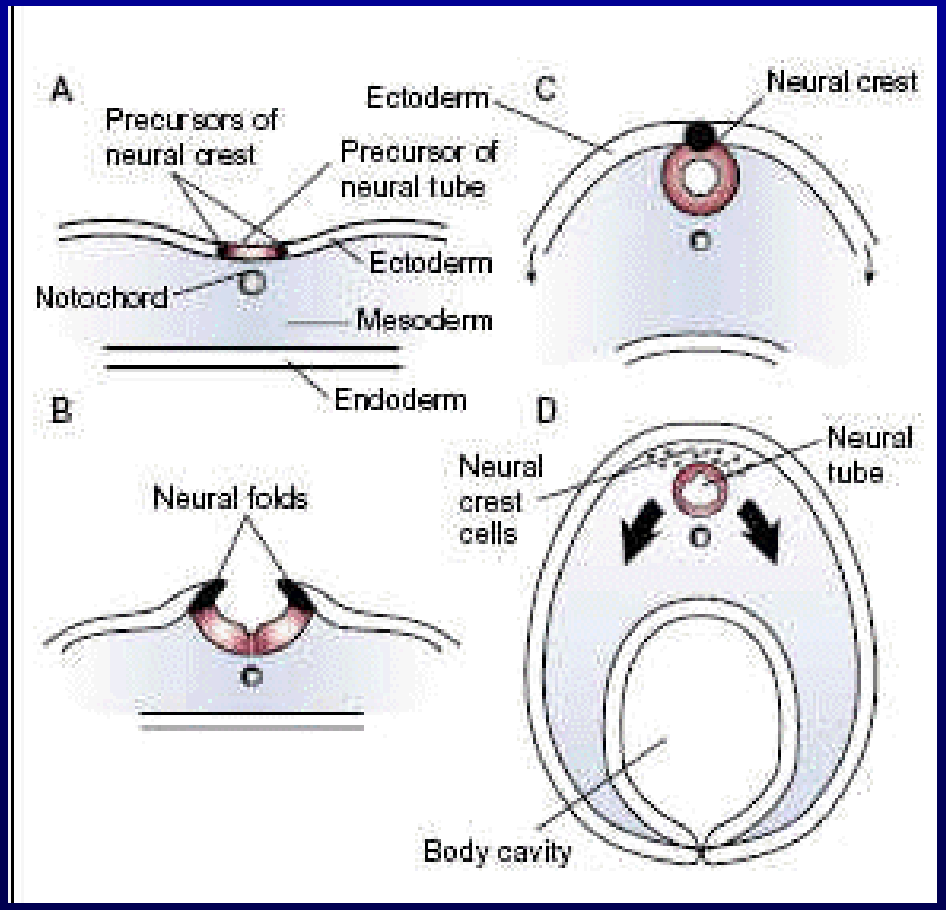
Formation of the Neural Tube



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

	NTD	Controls	P Value
RCF (ng/mL)	141	228	< 0.001
Vitamin C (WBC)	23.9	34.5	< 0.05

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

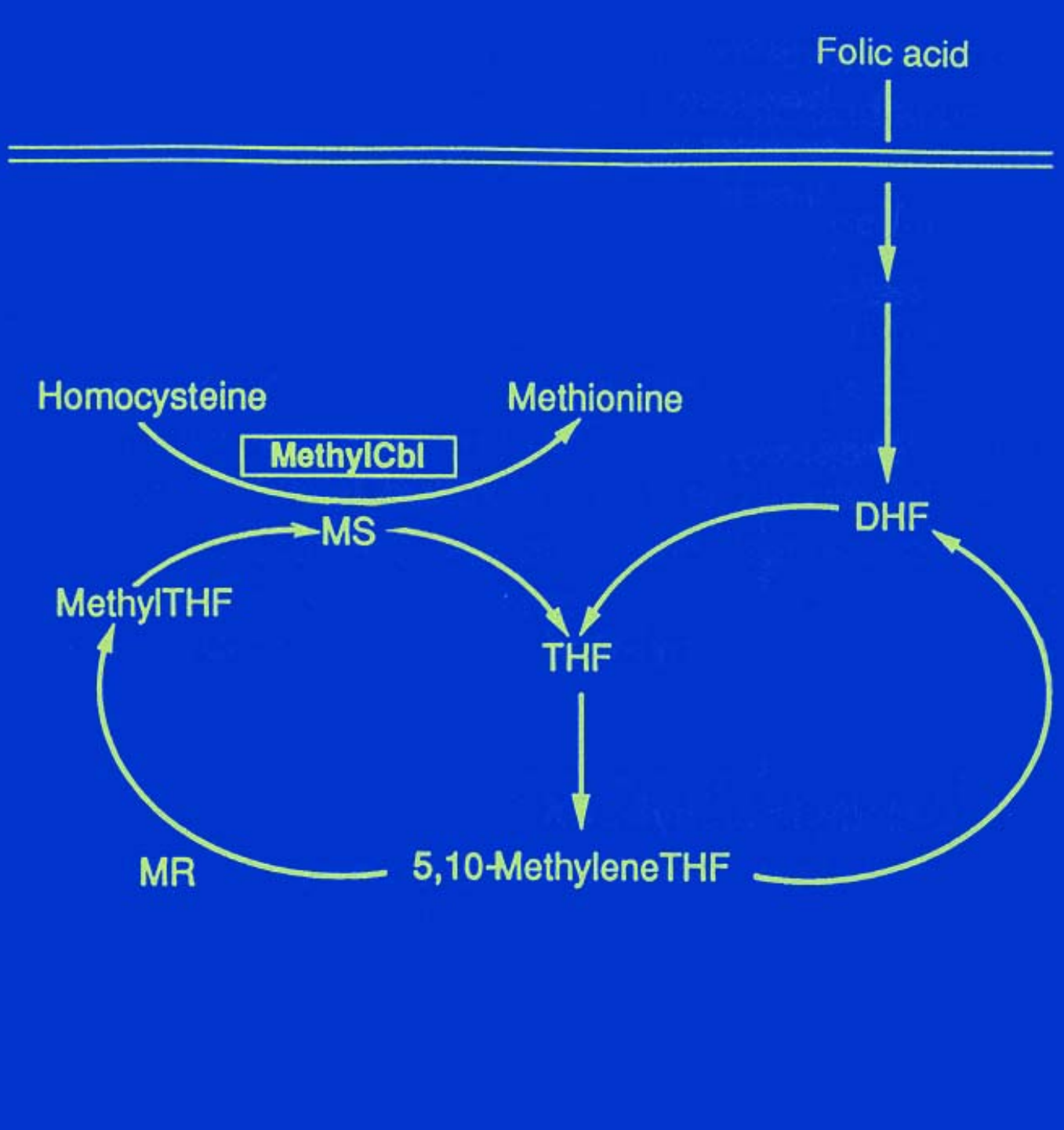
Cases	3.52 ± 3.1 µg/L
Random Controls	4.54 ± 4.3 µg/L

P = 0.004

Plasma B12

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

P = 0.008

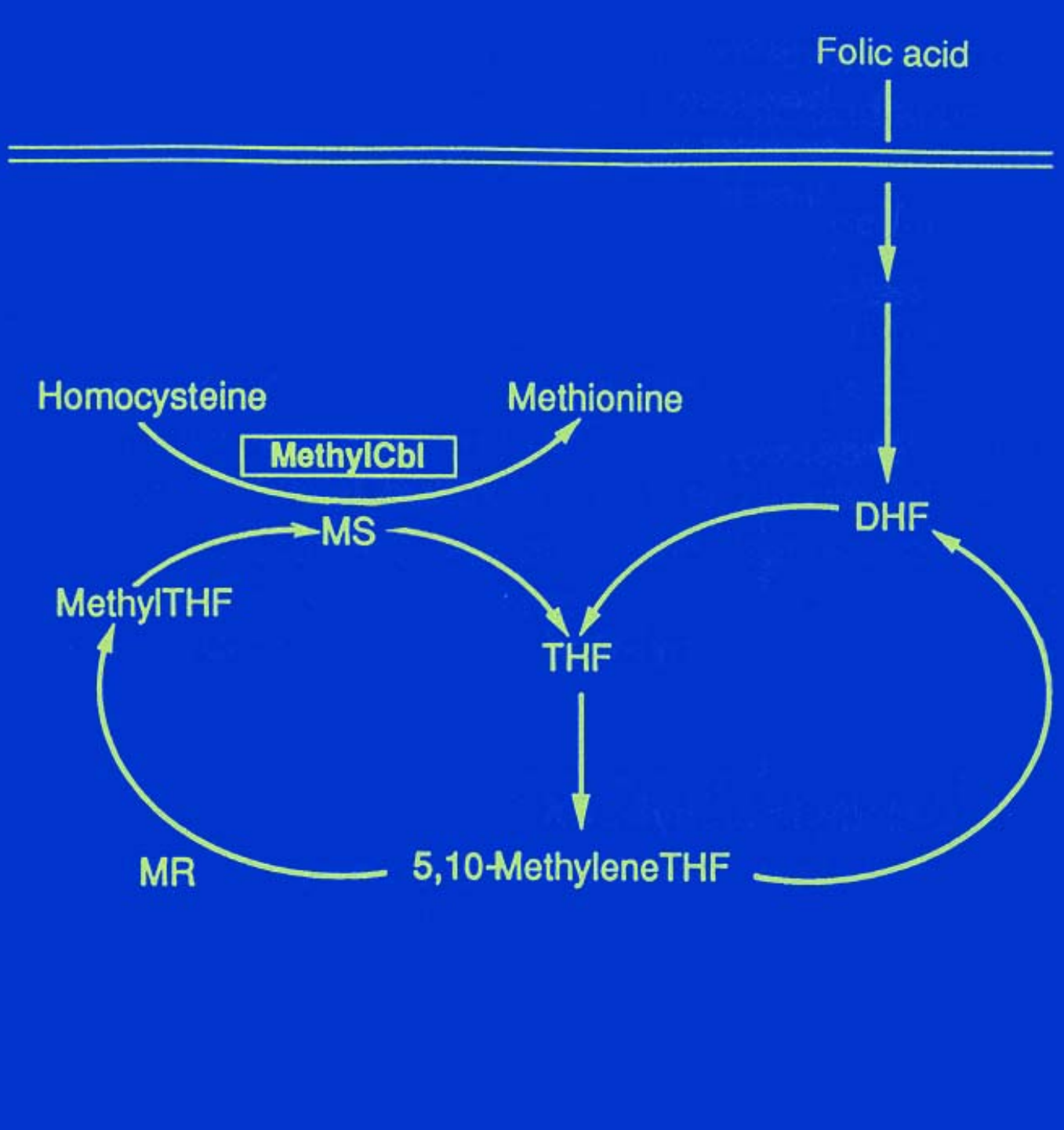

Folic acid
Homocysteine
Methionine
MethylCbl
MS
DHF
MethylTHF
THF
MR
5,10-MethyleneTHF

Homocysteine

Cases	8.62 ± 2.8 µmol/L
Combined Control Groups	7.96 ± 2.5 µmol/L

P = 0.03

What is the genetic problem?


Folic acid
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THF
MR
5,10-MethyleneTHF

5,10-Methylene Tetrahydrofolate Reductase (MTHFR) Study

- Thermolabile variant—MTHFR 677 C→T
- A → V substitution
- Reduced activity

MTHFR Genotypes in NTD Cases and Controls

	Cases N = 83	Controls N = 109
TT	18.3% (15)	6.1% (6)
CT	39.0% (32)	43.4% (43)
CC	42.7% (36)	60.5% (60)

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 ?

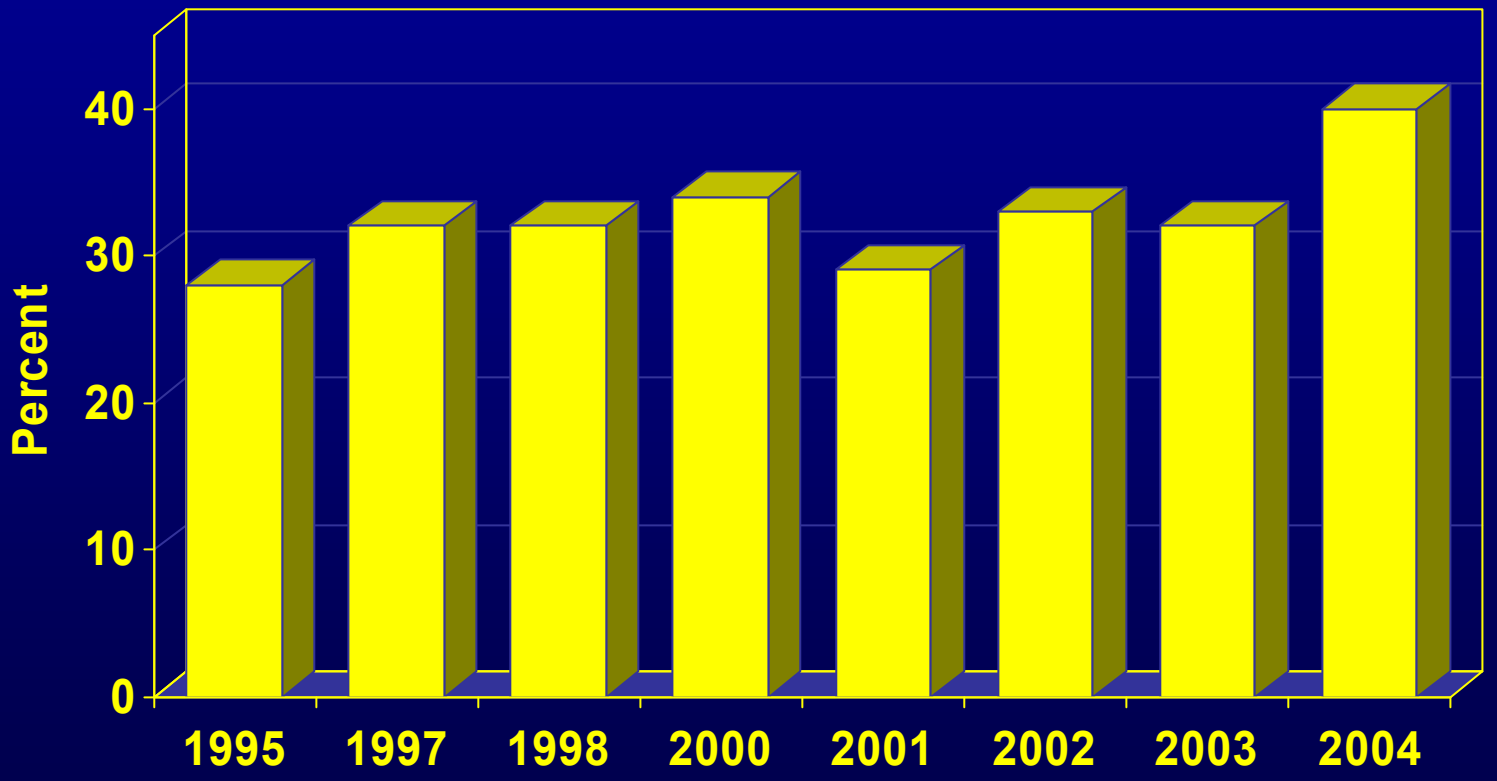
- 400 micrograms above current dietary intake requires supplement use or fortification
- Will people take supplements?

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

MTHFR Genotype	Mean RCF (µg/L)
CC	347
CT	314
TT	284*

Molloy AM, et al., *Lancet*, 1997; *$p<0.01$

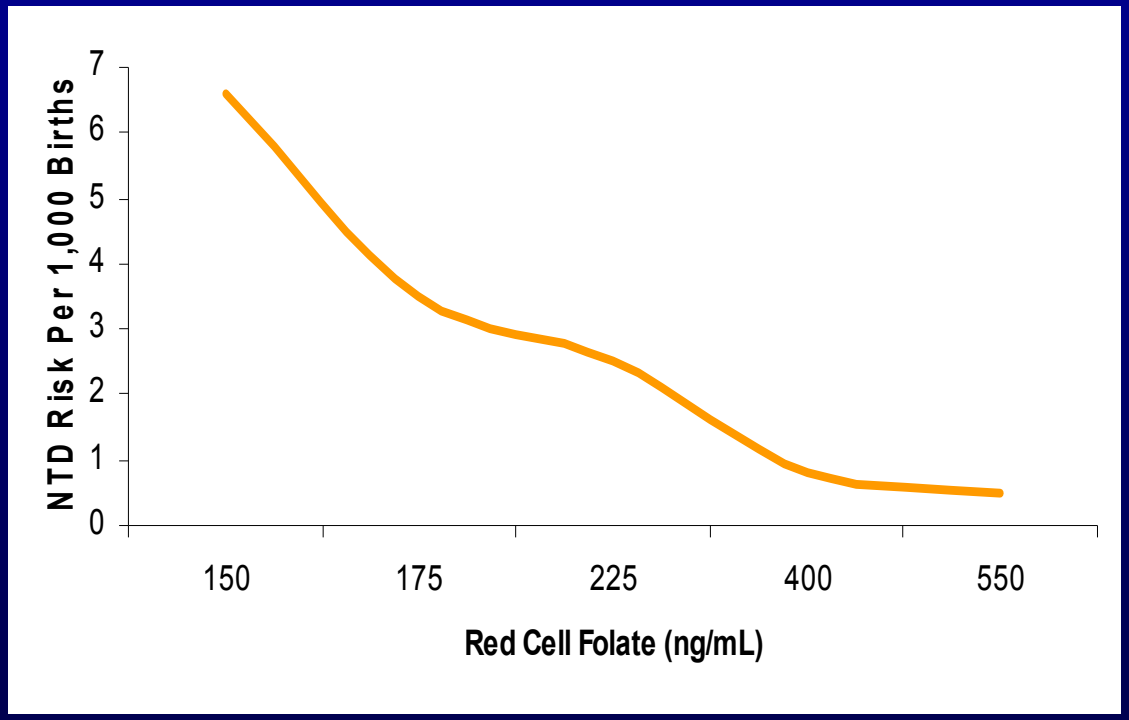
How Can We Estimate How Much Folic Acid is Needed?

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

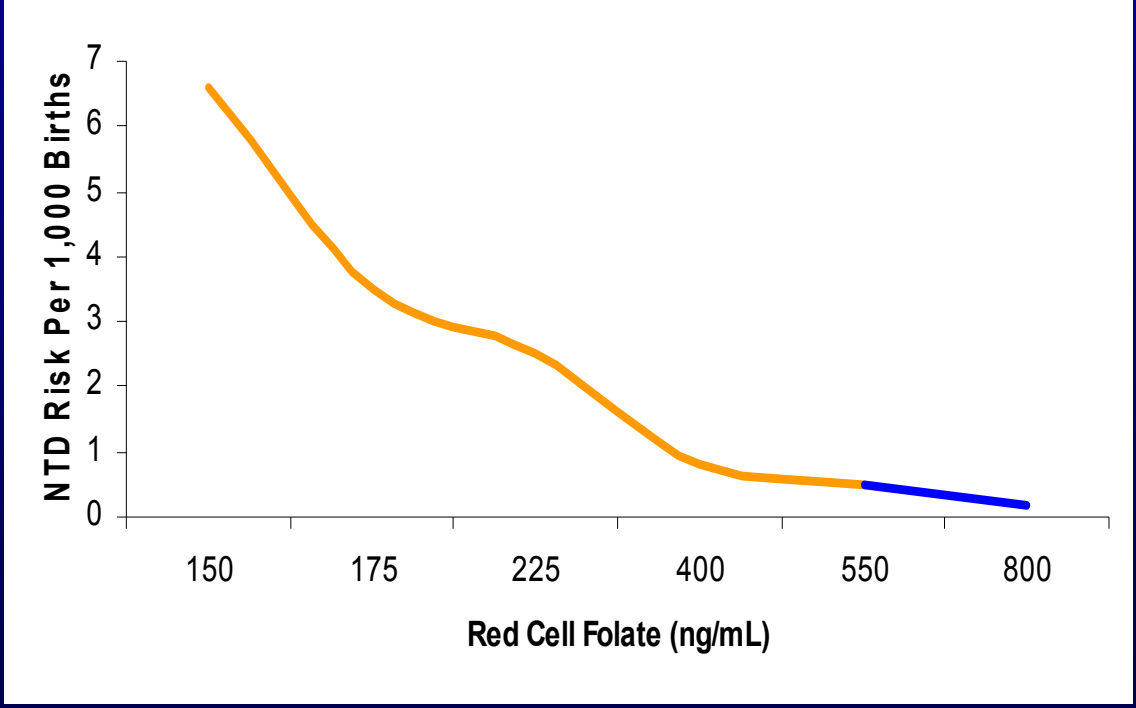
Maternal RCF vs. NTD Risk in Dublin



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

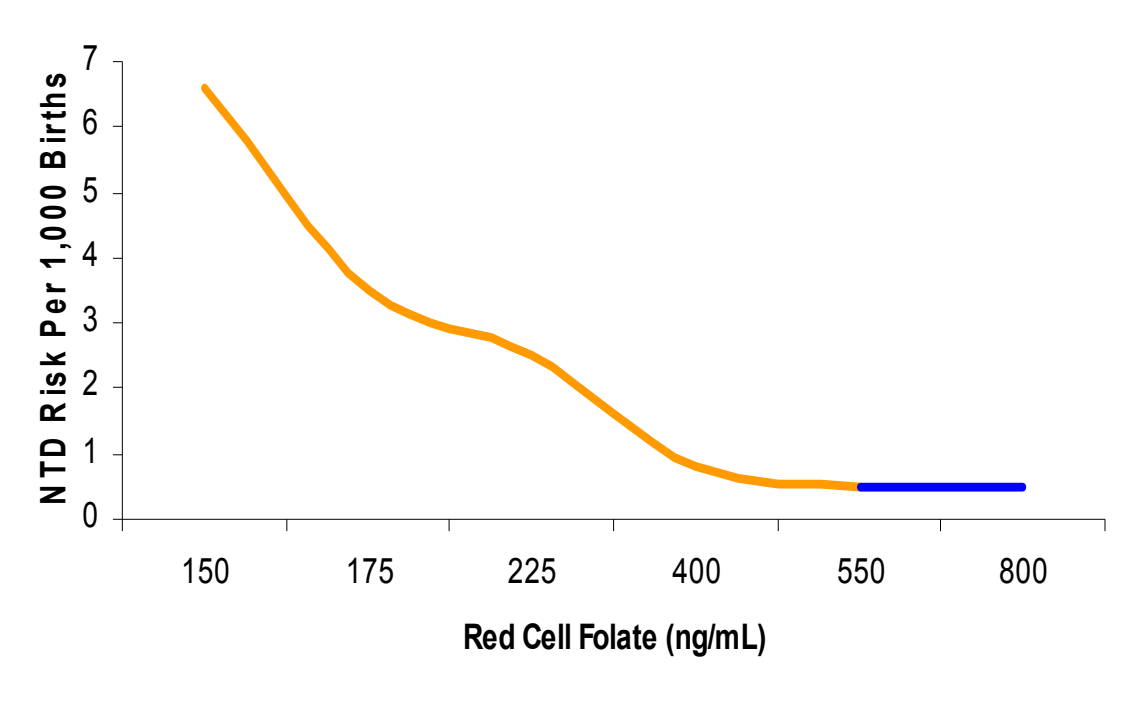
Data Source: Daly L, et al. Folate levels and neural tube defects. Implications for prevention. *JAMA* 1995;274:1698-702.

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 → 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

Daly S, et al., *Lancet*, 1997

Results

Group	N	Initial Median RCF µg/L	Final Median RCF µg/L	Median Change µg/L
Placebo	19	335	311	-12
100 µg/d	22	309	375*	67
200 µg/d	28	311	475*	130
400 µg/d	26	350	571*	200

Daly S, et al. *Lancet*, 1997 * $p < 0.001$

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

Daly S, et al. *Lancet*, 1997

Results

Treatment Group	N	Estimated Reduction in NTD Risk
Placebo	19	0
100 µg/d	22	22%
200 µg/d	28	41%
400 µg/d	26	47%

Daly S, et al. *Lancet*, 1997

Minimum estimate
(considering non-compliance):

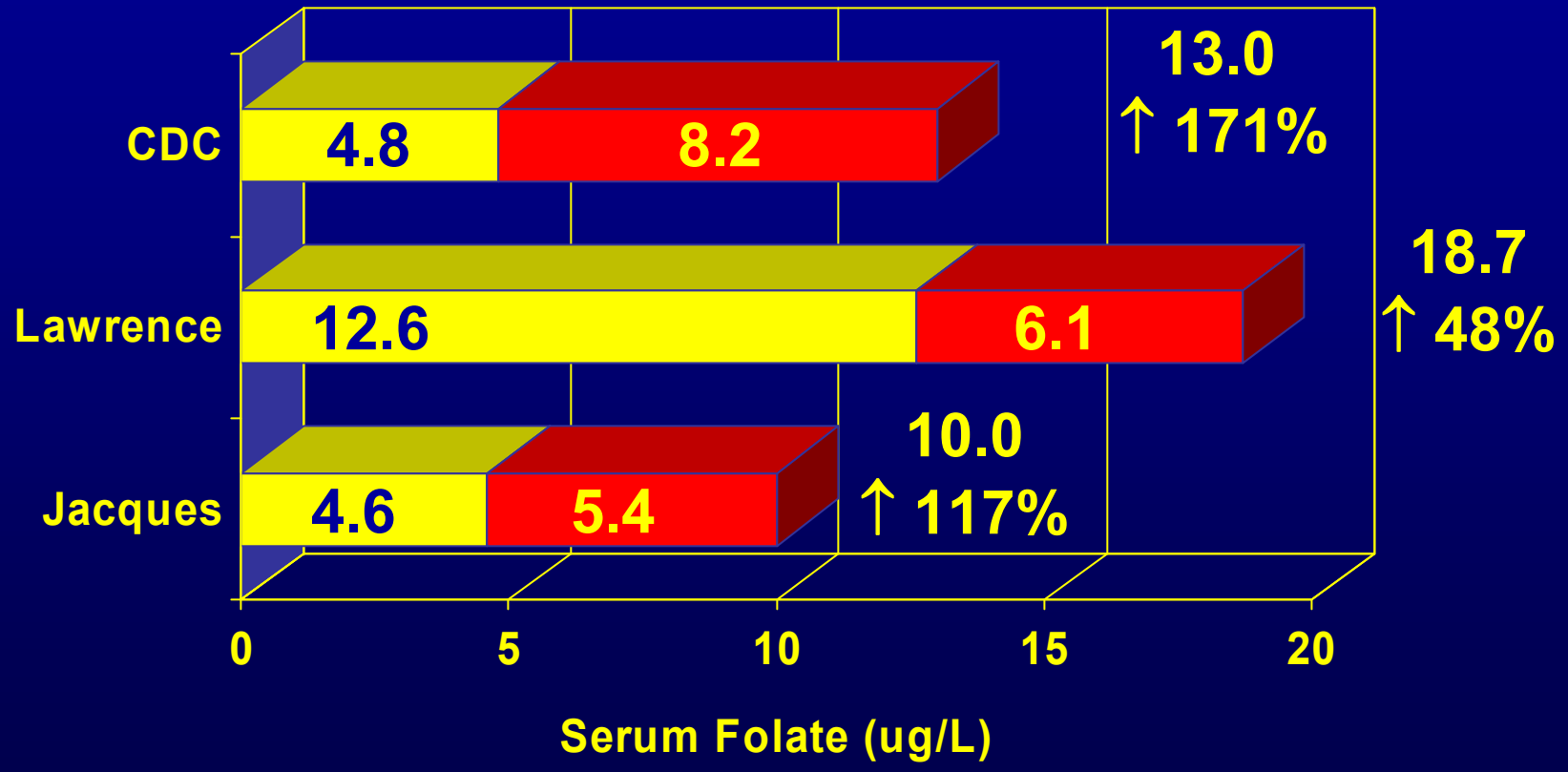
~200 µg/d will
decrease NTD rate by 41%

How Has Fortification Affected Folate Levels?

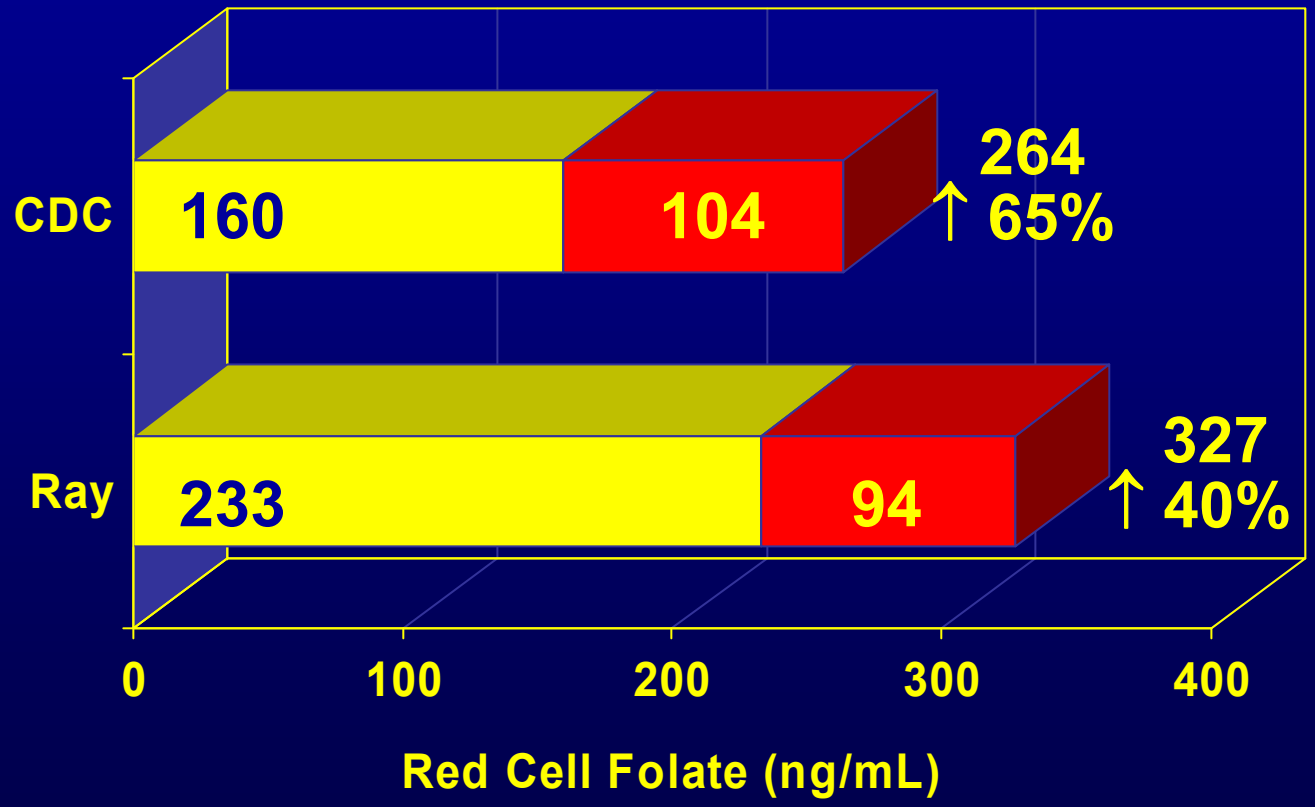
Was the FDA estimate correct?

- 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



RCF Levels
Pre- and Post-Fortification



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

Method of Detection	Percent
Ultrasonography	55%
MSAFP	25%
AF AFP	3%
Delivery	17%

Stevenson RE, et al., *Pediatrics*, 2000

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 → 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_{12} Deficiency

- Problem in the elderly
  - ≥ 12% of elderly in Framingham cohort are B12 deficient
- Protean neurologic signs make diagnosis difficult

Lindenbaum J, et al. *Am J Clin Nutr,* 1994

How great is the risk for masking?

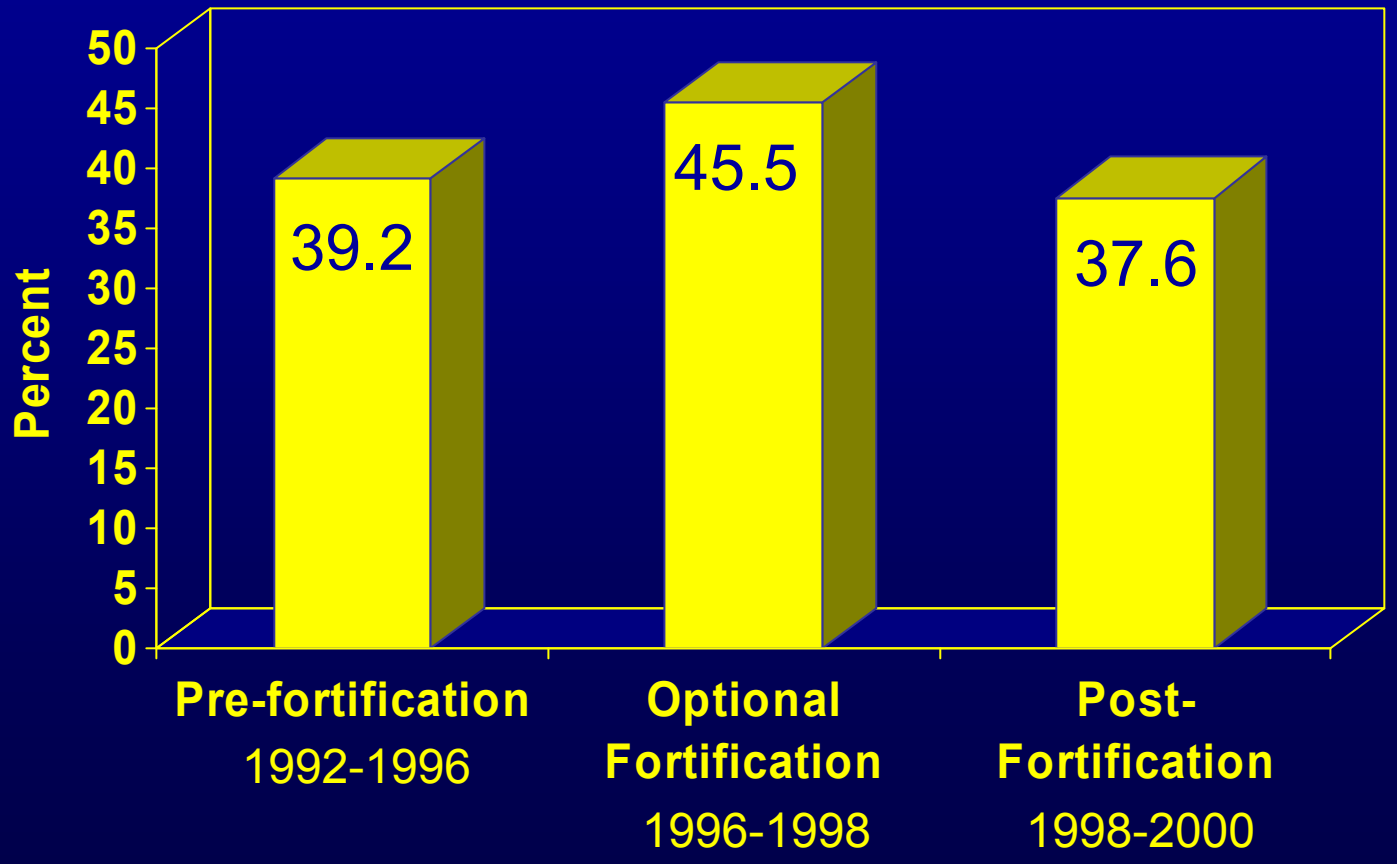
- The only data come from old, anecdotal reports
- Has been seen at doses as low as 400 µg/day
- Does not always occur at very high doses
- Collecting more data would be unethical

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

Mills JL, et al. *Am J Clin Nutr,* 2003

Proportion of Subjects With Low B_{12} Without Anemia



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_{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

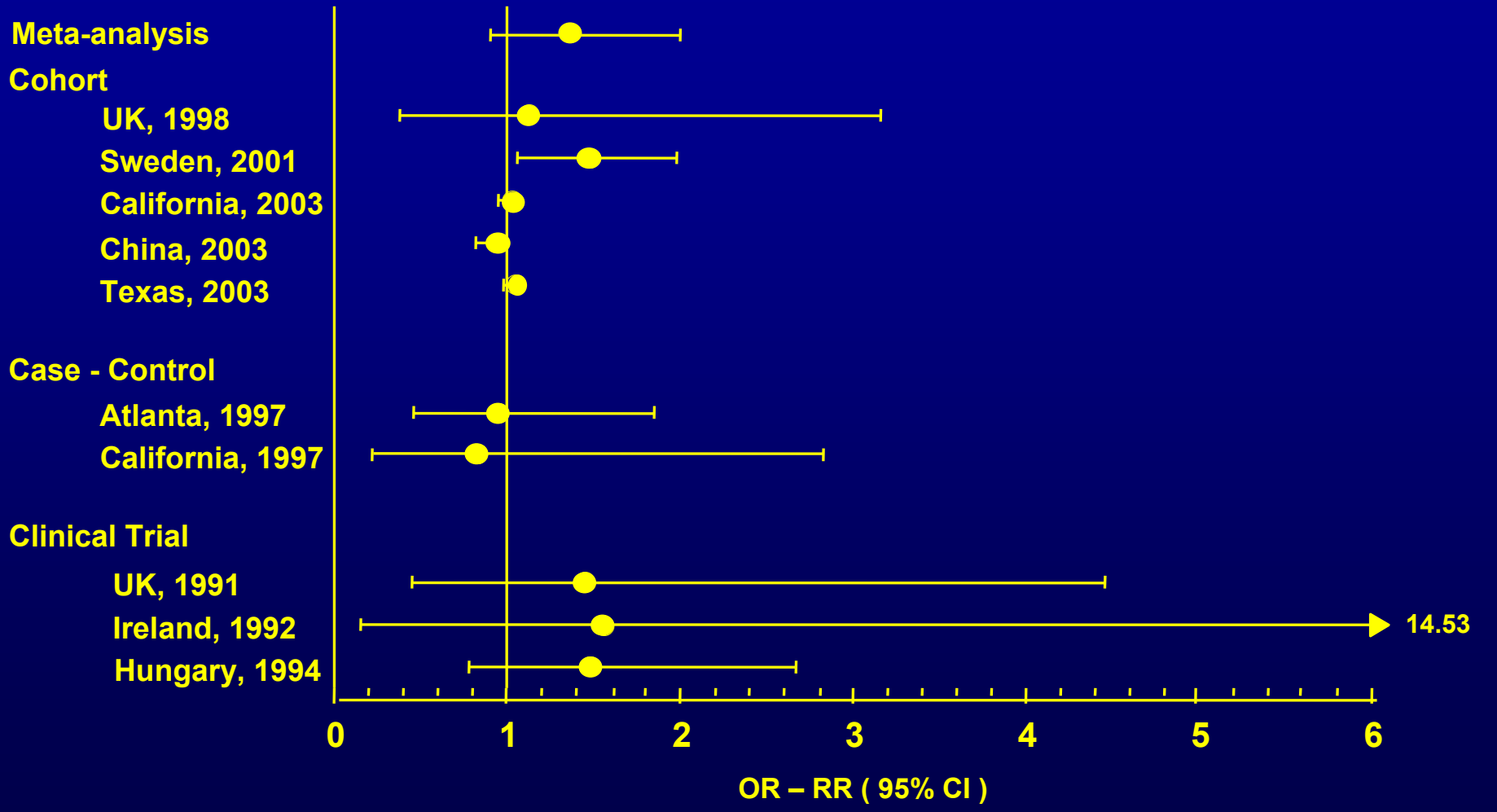
Czeizel AE, et al. *Acta Genet Med Gemeliol*, 1994

Number and Rate of Multiple Gestations

	Folic Acid/MV Group	Trace Element Group	P
Births	89/2243 (3.97%)	58/2199 (2.64%)	0.01
Pregnancies	44/2198 (2.00%)	29/2170 (1.34%)	0.09

Czeizel AE, et al. *Acta Genet Med Gemeliol*, 1994

Folic Acid and Multiple Gestation: Data Summary



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%) v. 0/9 (0%), p = 0.02

Takacs P, et al., *J Reprod Med*, 2004

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, O_2
- No comparison population for studies

Summary

- Masking B_{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