



# Interpreting folate status with biomarker and intake information from NHANES

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*National Conference on Health Statistics,  
August 16-18, 2010 --- Washington, DC*

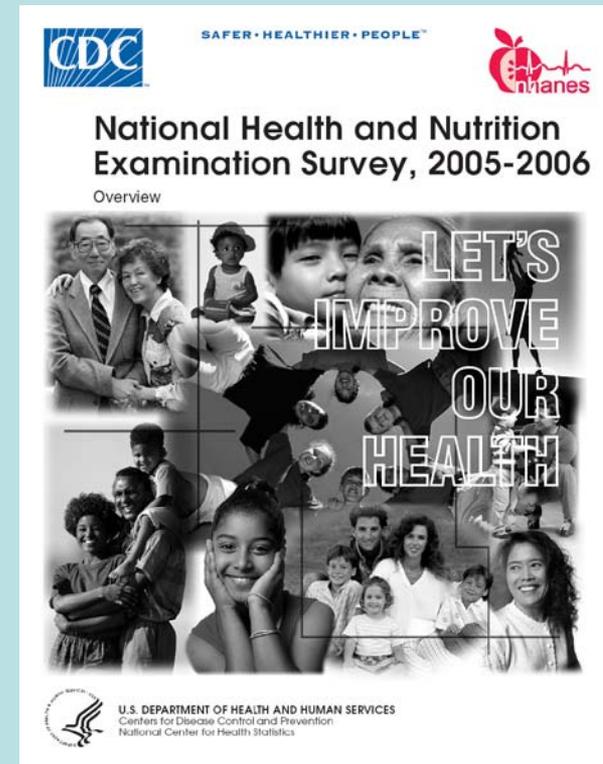
# Presentation outline

- Tools available in NHANES for nutritional status assessment
- Folate fortification policy
- Interpreting folate status using biomarkers and dietary intake information from NHANES
  - Strengths and weaknesses or challenges
- Lessons learned and way forward

# US NHANES

## Cross-sectional nationally representative survey

- Conducted by the National Center for Health Statistics, CDC
- Designed to collect information about the health and diet of people in the United States
- **Unique in that it combines a home interview with tests that are done in a Mobile Examination Center**



# NHANES tools for nutritional status assessment

## Dietary intake

Food frequency questionnaire (by mail)

Single 24-h dietary recall (MEC)

Second 24-h dietary recall by phone 3-10 days later (for all participants or subset only)

## Supplement usage

Dietary supplement questionnaire (household)

Use of dietary supplements over the past 30 days

Name/type; consumption frequency; duration of use; amount taken

Average daily intake of dietary supplement can be calculated

## Biomarkers

Blood draw and urine collection (MEC)

Specimen processing and aliquoting (MEC)

Specimen storage at -20°C until end of week (MEC)

Specimen shipment on dry ice to testing laboratory





# DIETARY INTAKE DATA

*What We Eat in America*

NHANES 2005-2006

- Dietary intake interview component of NHANES, conducted as partnership between USDA and DHHS
  - Replacing the previous USDA Continuing Survey of Food Intakes by Individuals (CSFII)
  - Intake of nutrients from foods estimated using the most current USDA Food and Nutrient Databases for Dietary Studies (FNDDS)

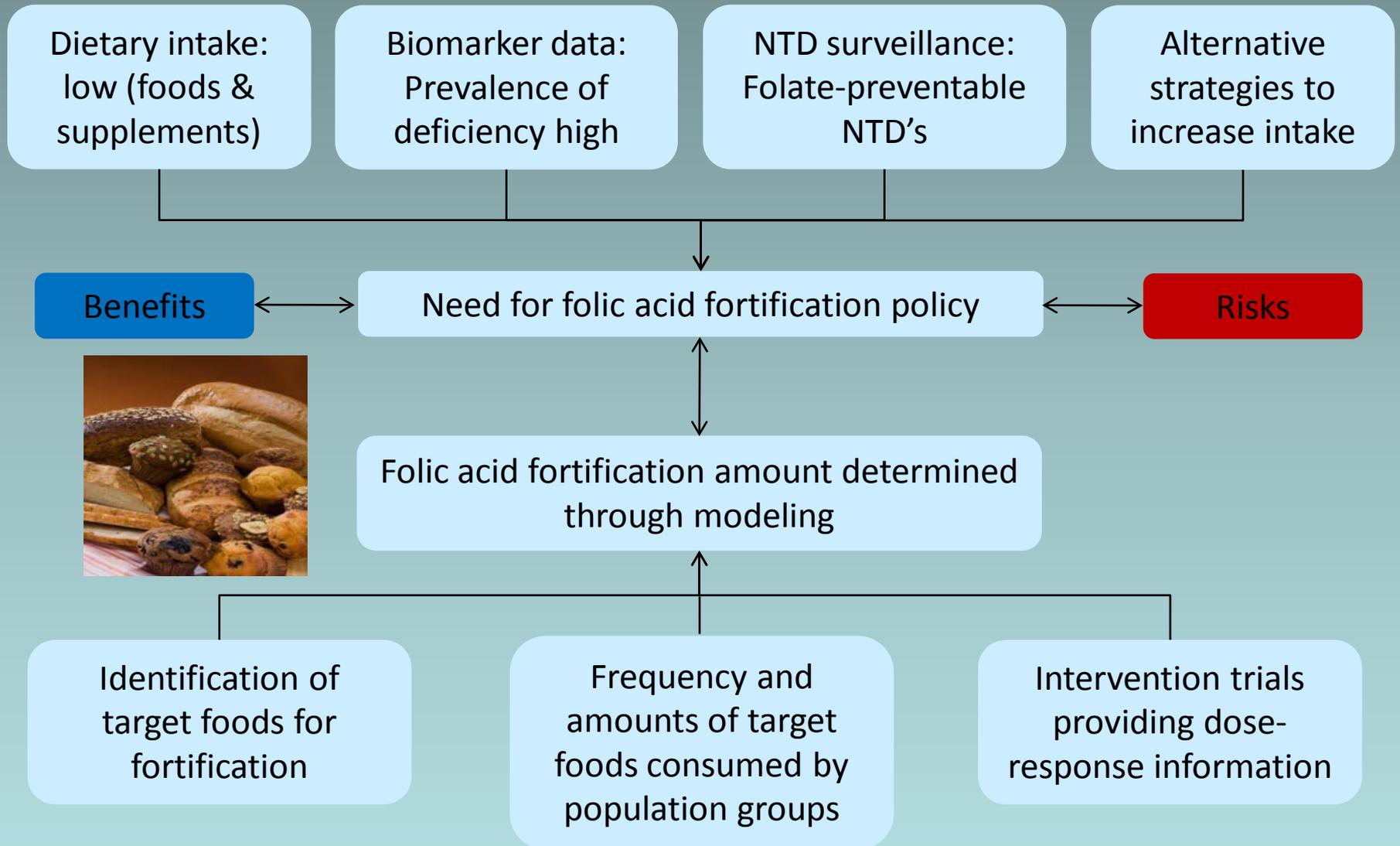


# National Report on Biochemical Indicators of Diet and Nutrition in the U. S. Population 1999-2002

[www.cdc.gov/nutritionreport](http://www.cdc.gov/nutritionreport)



# Introduction of folic acid fortification



# Association of folate with health outcomes

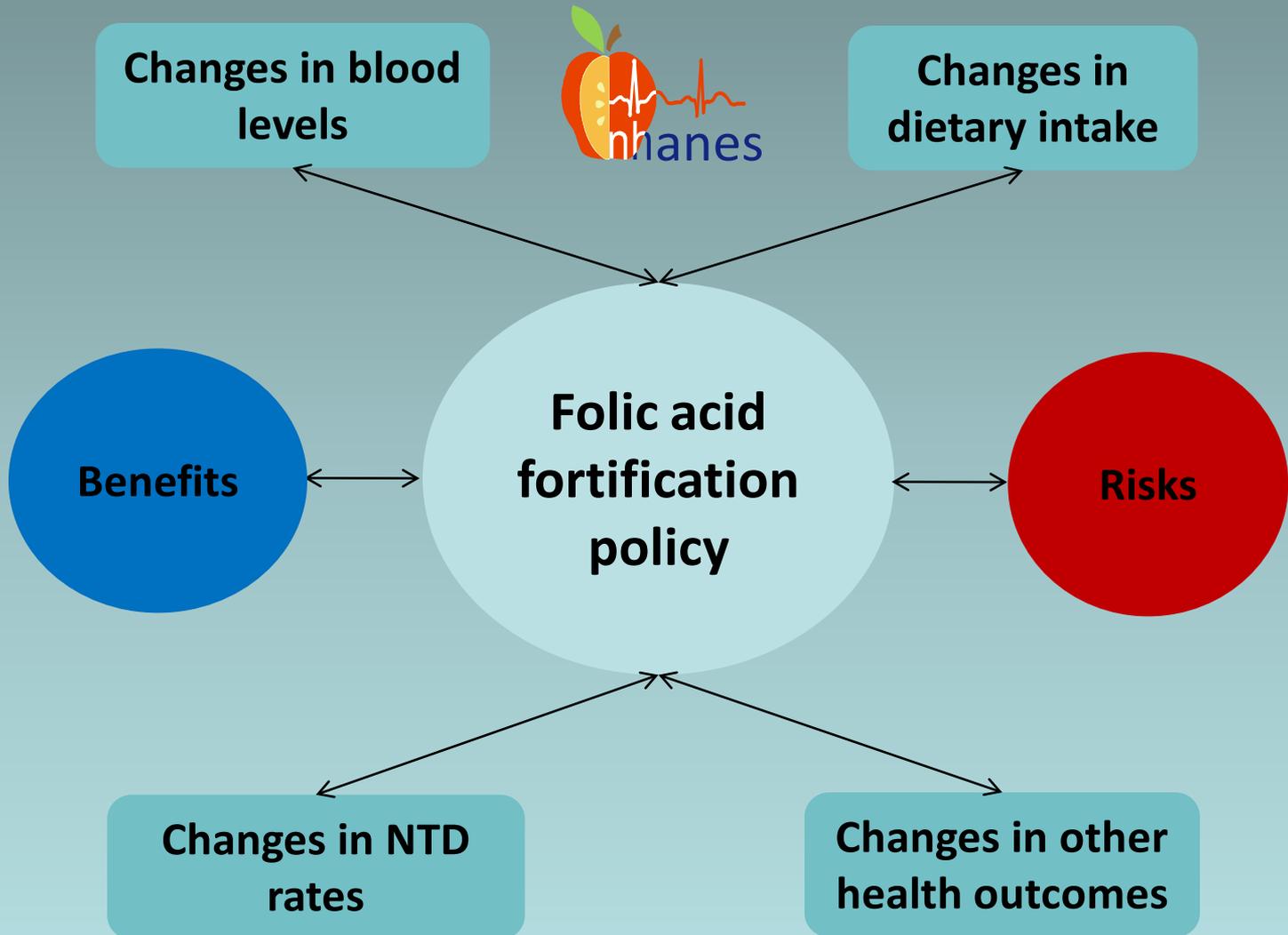
- NTD's and other birth defects
- Cardiovascular disease
- Cognition
- Cancer
  
- Acceleration of cancerous growth
- Masking of vitamin B12 deficiency
- Twinning
- Immunity
- Epigenetic changes

**Proven effectiveness of folic acid intervention**

**Cause and effect has not been proven**

**Potential adverse effects; basis is observational data**

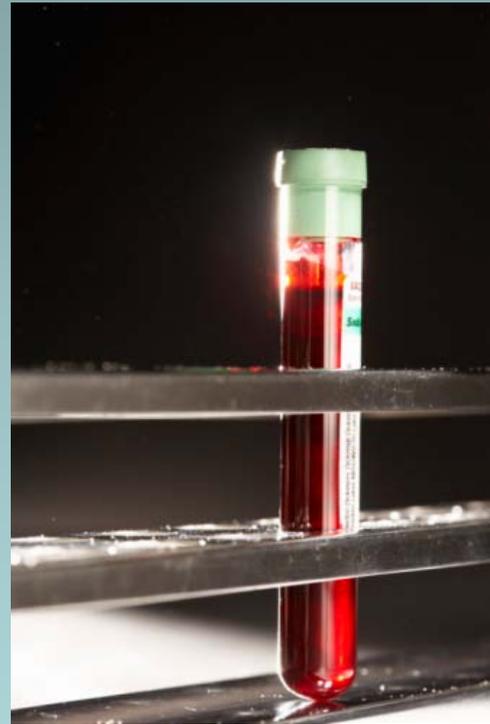
# Monitoring of the impact of folic acid fortification



# Changes in biomarker levels of folate status

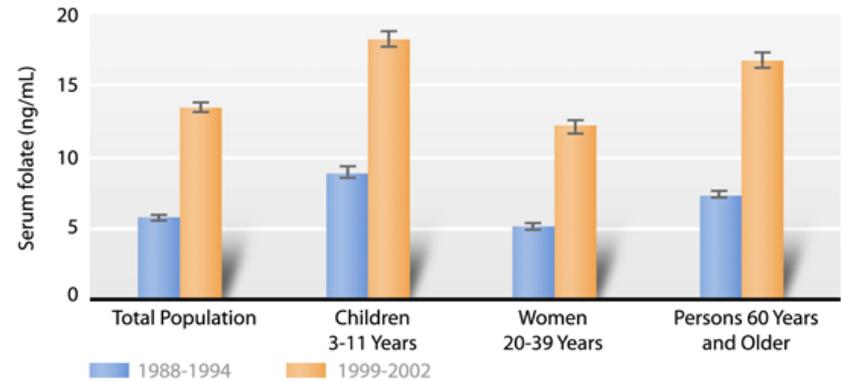
How much did folate blood levels change after the introduction of fortification?

What are the challenges associated with assessing folate status through biochemical measurements?



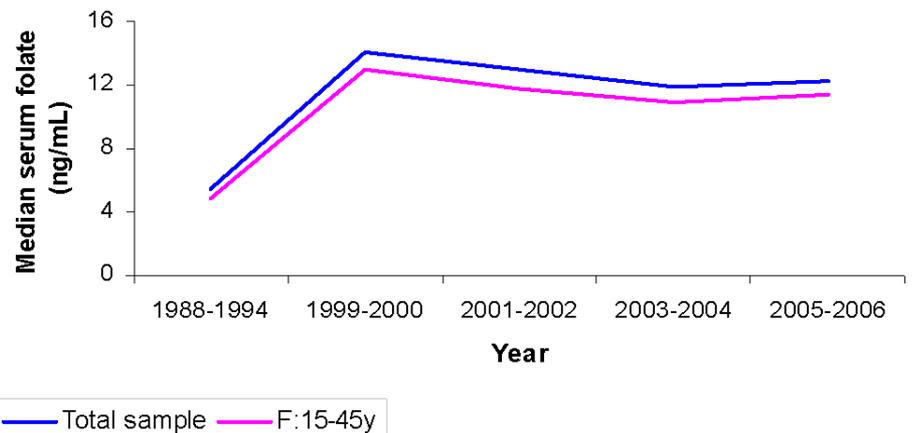
# Serum folate levels have nearly tripled

- Serum folate levels have increased much more than expected from FDA intake modeling and short-term FA supplementation trials – demonstrating the value of biomonitoring.



<http://www.cdc.gov/nutritionreport>

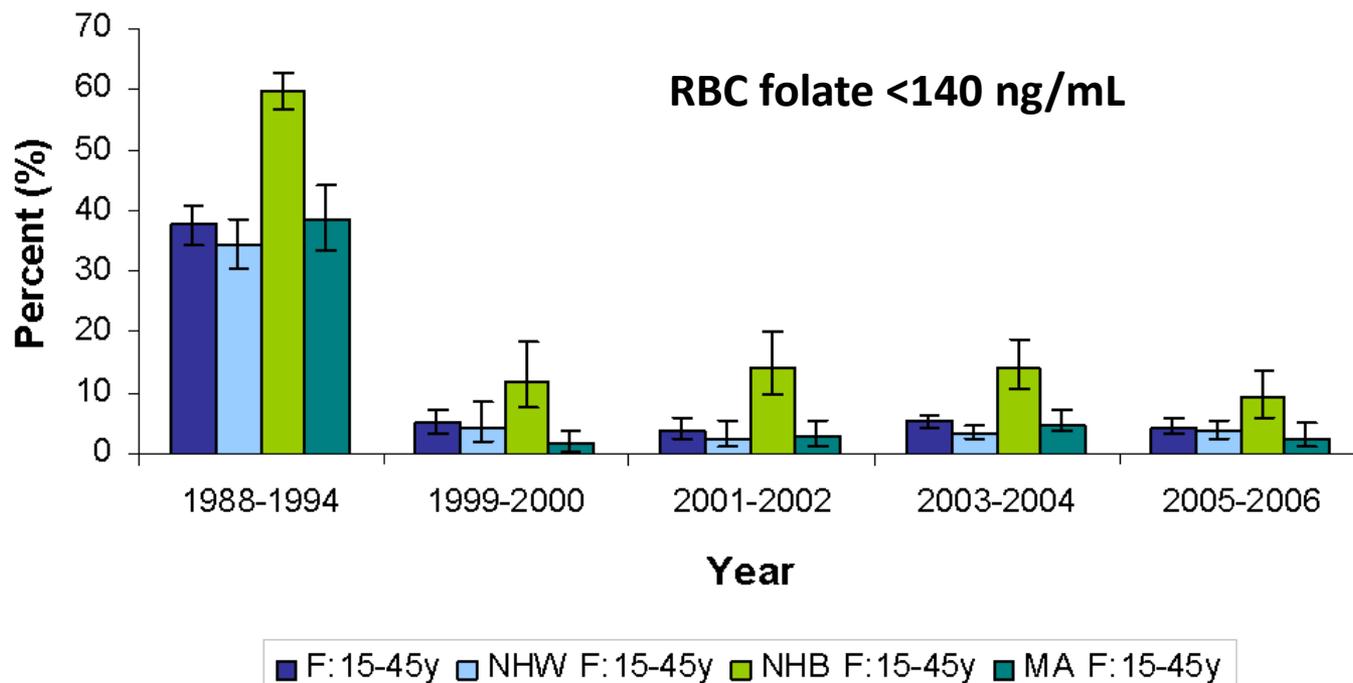
- Post-fortification serum folate levels have stabilized after several years.



<http://www.cdc.gov/nchs/data/databriefs/db06.htm>

# Prevalence of low RBC folate levels has decreased

Red blood cell folate levels have also stabilized after fortification and the prevalence of low levels in women of childbearing age was ~5% compared to ~40% at pre-fortification.



<http://www.cdc.gov/nchs/data/databriefs/db06.htm>

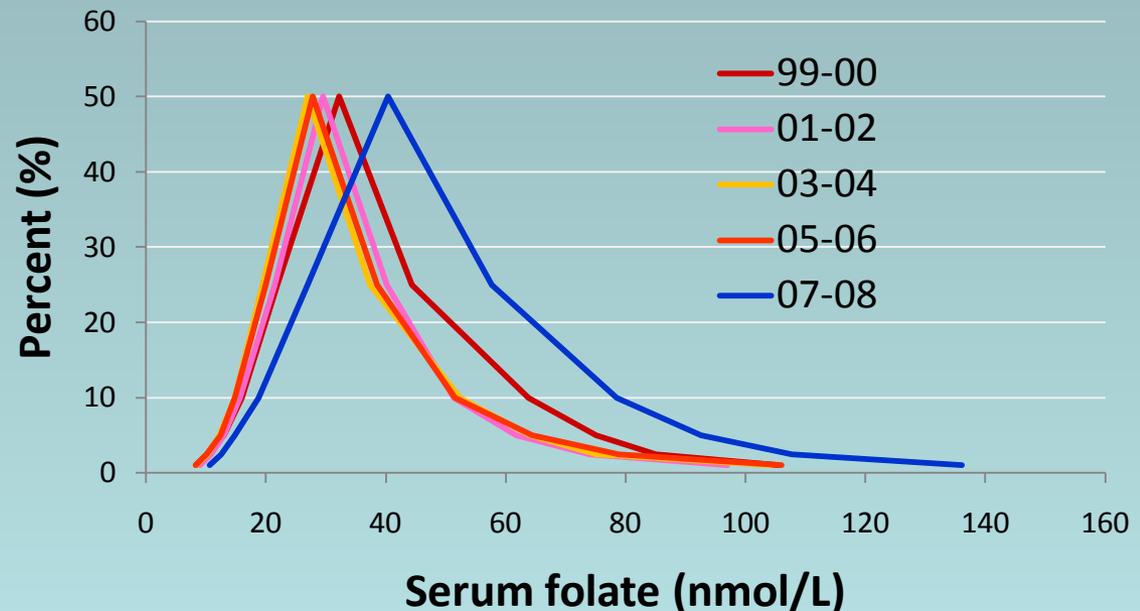
# Strengths of folate biomarker data

- Good reflection of nutritional status; low levels have been linked to functional outcomes (NTD's)
- The same assay (Bio-Rad RIA) was used from NHANES III to NHANES 2005-2006 to measure serum and RBC folate levels
- Excellent assay precision; small fluctuations over time can be detected
- Assay performance was stable over time; changes in population levels could be interpreted unequivocally



# Challenges of folate biomarker data

- Collection of blood is invasive; lab analyses are expensive
- Lack of folate assay standardization
  - Cutoff values of deficiency are method dependent
  - Difficulty comparing data between methods and countries
- Switch to new assay (microbiological assay) in 2007; requires cross-over data



# Changes in folate dietary intake

**How much did folate intake change after the introduction of fortification?**

**What are the challenges associated with assessing dietary intake of folate?**



# Sources of folate intake

Folate sources	Folate	Folic acid (FA)	Dietary Folate Equivalents (DFE)
Food (natural)	+	-	1 DFE = 1 $\mu\text{g}$ food folate
Food (fortified): ECGP + RTE cereals	+	+	1 DFE = 1 $\mu\text{g}$ food folate or 0.6 $\mu\text{g}$ FA from fortified food
Supplements	-	+	1 DFE = 0.6 $\mu\text{g}$ FA taken with food or 0.5 $\mu\text{g}$ FA on empty stomach



# Changes in folate dietary intake

## NHANES 1988-1994 versus 1999-2000, entire population:

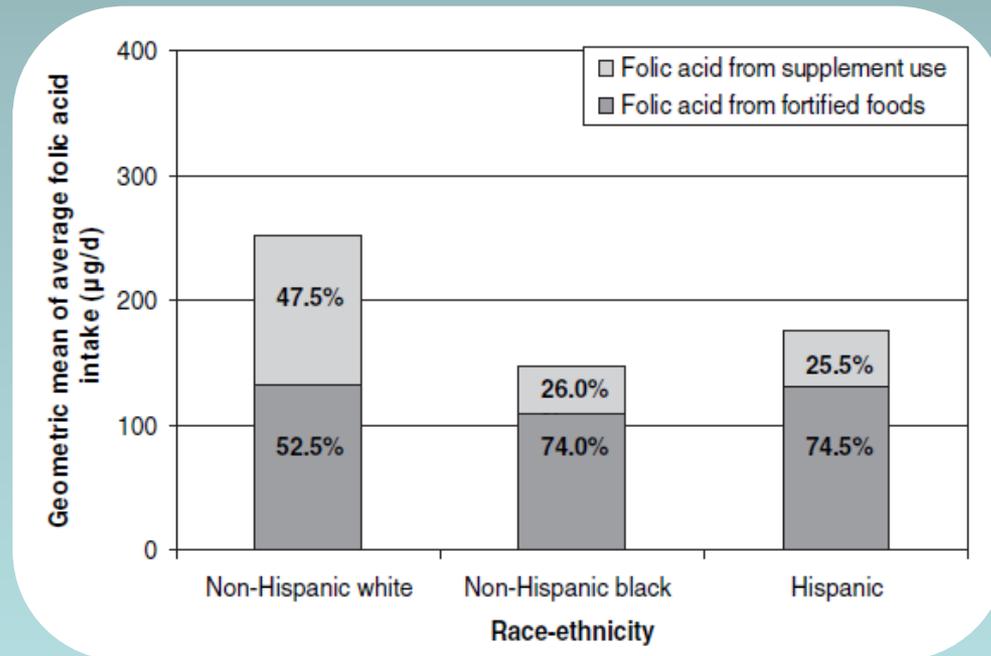
- After fortification, the category “**bread, rolls, and crackers**” became the single largest contributor of total folate to the American diet (15.6% of total intake), surpassing vegetables, which were the number one folate food source prior to fortification.
- The mean dietary total folate intake of the population increased by 76  $\mu\text{g}/\text{d}$  (28%), from 275  $\mu\text{g}/\text{d}$  to 351  $\mu\text{g}/\text{d}$ .
- **Caveat:** The dietary folate data in the NHANES nutrient database are reported as total folate intake (food folate + folic acid from fortification) in  $\mu\text{g}/\text{day}$ , not as DFE, which takes the higher bioavailability of folic acid compared to food folate into account (1 DFE = 1  $\mu\text{g}$  food folate = 0.6  $\mu\text{g}$  folic acid from fortified food).

*Dietrich M et al. J Am Coll Nutr 2005*

# Post-fortification total folic acid intake

**NHANES 2001-2002, women of childbearing age:**

- Neither race/ethnic group consumed the recommended 400  $\mu\text{g}$  of folic acid daily from fortified food and/or supplements.
  - The average estimated daily total folic acid intake was 221  $\mu\text{g}$
- Caveat:** Underestimation by  $\sim 10\%$  - no second 24-h dietary recall.

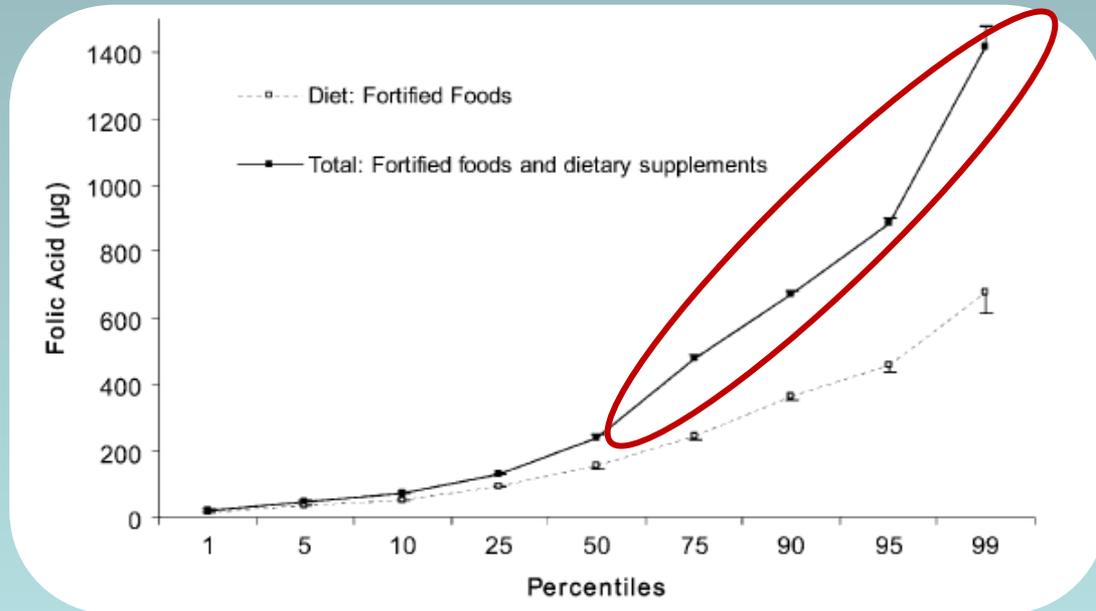


*Yang Q-H et al. AJCN 2007*

# Post-fortification total folate and folic acid intake

## NHANES 2003-2006, entire population:

- 14-19% of women and 5.2-9.2% of men had a folate intake below the EAR (320  $\mu\text{g}/\text{day}$  of DFEs).
- For those consuming high amounts of folic acid, the folic acid comes from dietary supplements, not from fortified foods



Bailey RL et al. AJCN 2010

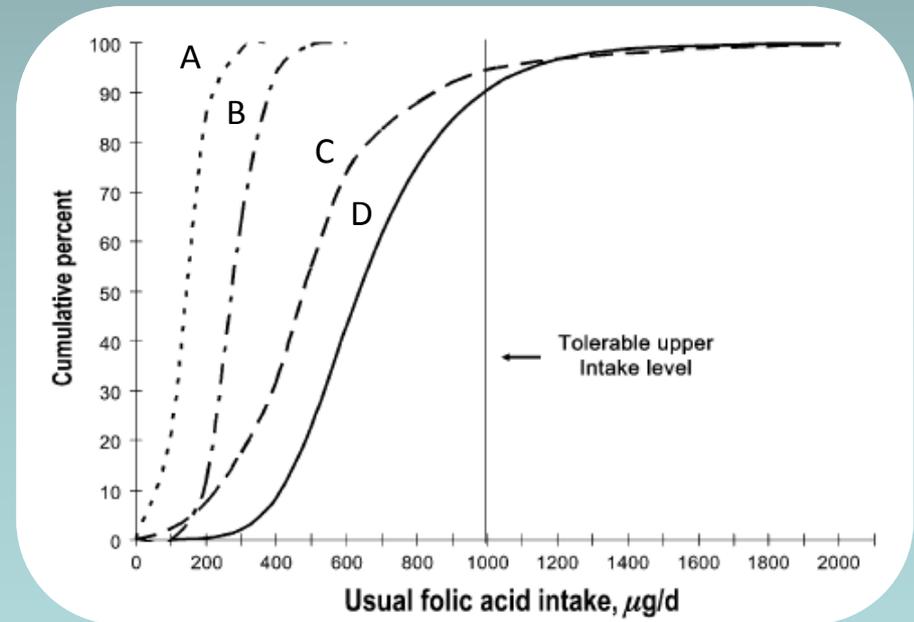
# Post-fortification folic acid source, usual intake and UL

## NHANES 2003-2006, entire population:

- Of the total US adult population, 2.7% exceeded the UL for folic acid (1,000  $\mu\text{g}/\text{day}$  of folate from fortified food or as a supplement, exclusive of food folate).

Folic acid source	Exceeding UL
A. ECGP only	0%
B. ECGP + RTE	0%
C. ECGP + SUP	5.5%
D. ECGP + RTE + SUP	9.4%

ECGP = enriched cereal grain products  
RTE = ready-to-eat cereals  
SUP = dietary supplements



Yang Q-H et al. AJCN 2010

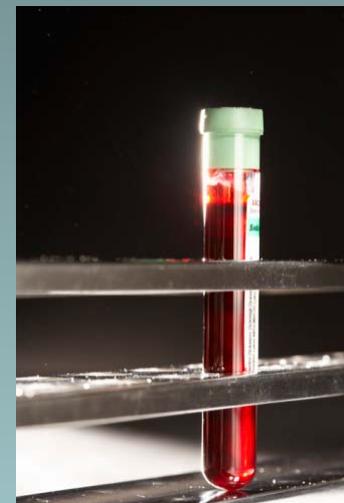
# Folate dietary intake data

Strengths	Challenges
Non-invasive	Self-reported data; flawed with multiple errors
Relatively easy and inexpensive to conduct	Various sources of intake need to be captured
Easier to compare between countries	Computation of data is complex (DFE)
	Requires two 24-h dietary recalls to calculate usual intakes



# Summary - US folic acid fortification intervention

- Biomarker levels have responded as expected:
  - Right direction
  - But, much bigger magnitude
- Dietary intake levels have also responded as expected:
  - Right direction
  - But, folate intake is still below the EAR for a small portion of the population
  - Women of childbearing age still don't consume on average the recommended 400  $\mu\text{g}$  of folic acid daily
  - Only a small fraction of those who consume supplements exceed the UL of folic acid



***Which data tell us the truth?***

# Lessons learned from population-based surveys



# Requirements for nutritional biomarkers in population-based surveys

- Precision of assay to enable detection of small trends
- Robustness of assay to ensure comparability of data over time
- Continuity of same assay over time, when possible
- If assay changes occur, need cross-over study to enable data comparison over time
- Testing laboratory:
  - Internal quality control and regular assay verifications
  - External quality assurance
  - Reference materials
- Global community:
  - Assay standardization

# Data requirements to formulate and evaluate nutrition policy

- Nutritional biomarker data (multiple biomarkers where possible)
- Dietary intake data (usual intakes)
- Supplement usage information
- Demographic characteristics
- Behavioral factors
- Health indicators
- Coverage of various population groups
- Monitoring over multiple years

# WAY FORWARD

- NIH/ODS and NCHS sponsored roundtable on folate and vitamin B12 issues in NHANES in July 2010
- Future methodology to monitor serum folate levels should be LC-MS/MS
  - Highly specific higher-order reference methodology
  - Allows monitoring of free folic acid in addition to total folate
- If possible, microbiological assay should be continued to monitor RBC folate levels (at least in women of childbearing age)



# Acknowledgments



**Nutritional Biomarkers Branch**

- Donna LaVoie – Bio-Rad RIA
- Mindy Zhang – Microbiological assay
- Zia Fazili – LC-MS/MS
  
- Colleagues at NCHS
- Colleagues at NCBDDD
- Colleagues at NIH/ODS
- Colleagues at FDA

# Folate intake recommendations

- RDA (Recommended Dietary Allowance) for both men and women is **400 µg/day of DFEs** (dietary folate equivalents)
- EAR (Estimated Average Requirement) for both men and women is **320 µg/day of DFEs**
- To reduce the risk of NTDs for women capable of becoming pregnant, the recommendation is to take **400 µg folic acid daily** from fortified foods, supplements, or both in addition to consuming food folate from a varied diet
- UL (Tolerable Upper Intake Level) for adults is set at **1,000 µg/day of folate from fortified food or as a supplement**, exclusive of food folate

*Institute of Medicine, Food and Nutrition Board. Dietary reference intakes: Thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington, D.C.: National Academy Press; 1998.*