

Blood levels of folate over time, current US levels, and differences between assessment methods

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National Center for Environmental Health
Division of Laboratory Sciences



Disclosure

Nothing to disclose

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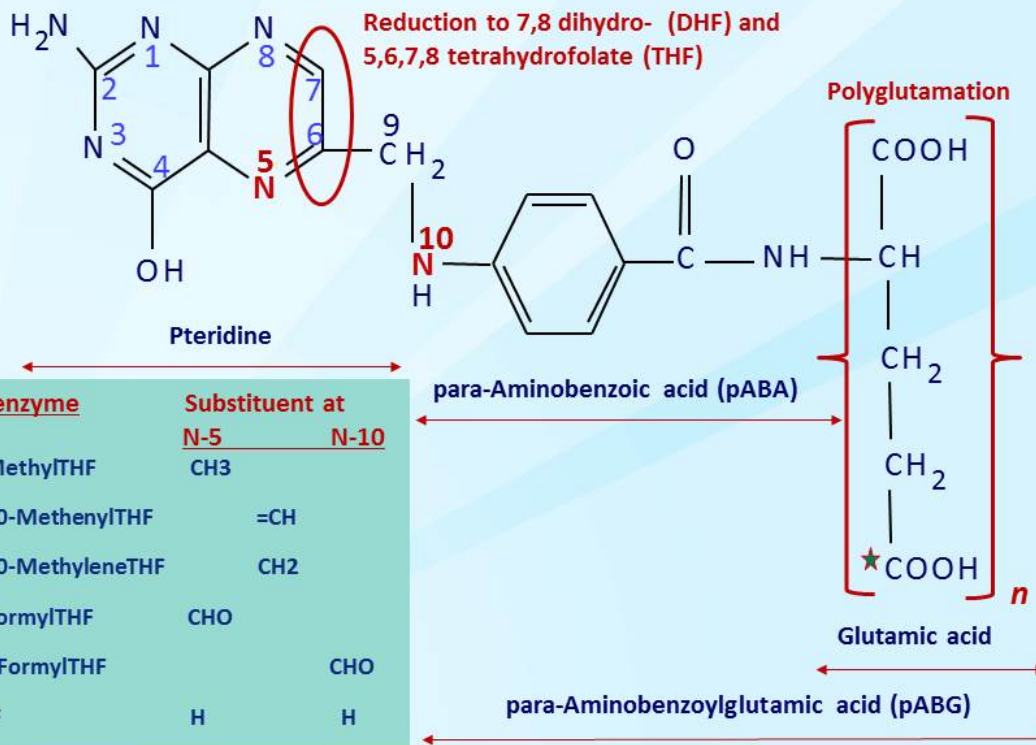
Presentation outline

- **Assays for serum and red blood cell (RBC) folate**
 - Brief overview of available methods
 - Issues with (lack of) comparability of data
 - Issues with cutoff values
- **Serum and RBC folate status pre- vs. post-fortification**
- **Current US blood folate levels**
 - Post-fortification concentrations of serum and RBC folate
 - Post-fortification prevalence estimates of low blood concentrations
 - Post-fortification concentrations of serum folate forms, including unmetabolized folic acid (UMFA)
 - Factors associated with higher UMFA concentrations

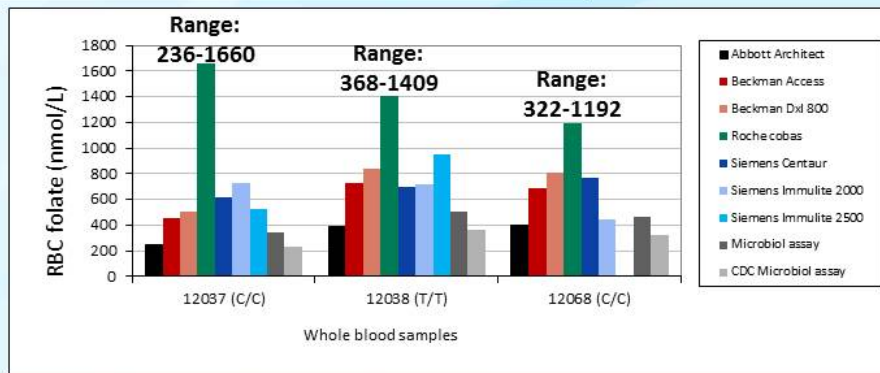
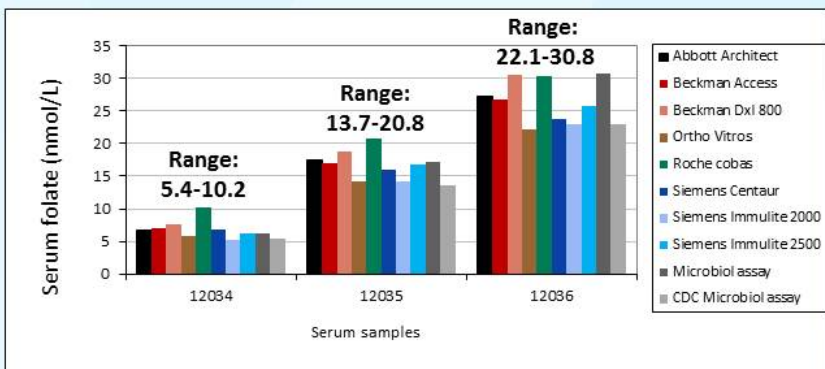
ASSAYS FOR SERUM AND RBC FOLATE



Folate structure allows variations in three areas



Serum and RBC folate – 2012 UK NEQAS PT program



Main laboratory methods for serum and RBC folate

| Method type | Advantages | Disadvantages |
|--|--|---|
| <u>Microbiologic assay</u> (MBA) for total folate | <ul style="list-style-type: none"> • Small sample volume • Inexpensive • Suited for low resource setting • Measures all biologically active forms approximately equally (however, calibration with 5-methylTHF generates lower results than calibration with folic acid) | <ul style="list-style-type: none"> • Relatively laborious and takes 2 days to result • Replicates needed due to higher imprecision • Multiple dilutions needed due to limited linear range • Inhibited by presence of antibiotics or antifolates |
| <u>Competitive protein binding assay</u> (CPBA) for total folate | <ul style="list-style-type: none"> • User friendly and minimum operator involvement • High sample throughput • Suited for clinical setting • Generally good precision (~5%) | <ul style="list-style-type: none"> • Questionable accuracy due to different affinities of folate forms to FBP • Less suited for long-term studies due to potential lot-to-lot variability • Matrix effects likely with sample dilution |
| <u>Chromatography-based assay</u> for folate (various detectors; recently MS/MS) | <ul style="list-style-type: none"> • Information on folate vitamers • Highly selective and specific • Good analytical sensitivity and precision • Suited for research setting | <ul style="list-style-type: none"> • Expensive instrumentation and technical service, experienced operator • Complex sample extraction/clean-up • Conversion of polyglutamates to monoglutamates needed for whole blood • Summation of folate forms to total folate |

Pfeiffer et al. – Folate methods chapter in Bailey's Folate in Health and Disease, 2nd ed.

Folate methods and data in NHANES

| NHANES | Serum folate | RBC folate |
|---------------------------|----------------|------------|
| Pre-fortification | | |
| 1988-1994 | BioRad RIA | BioRad RIA |
| Post-fortification | | |
| 1999-2000 | BioRad RIA | BioRad RIA |
| 2001-2002 | BioRad RIA | BioRad RIA |
| 2003-2004 | BioRad RIA | BioRad RIA |
| 2005-2006 | BioRad RIA | BioRad RIA |
| 2007-2008 | MBA (LC-MS/MS) | MBA |
| 2009-2010 | MBA | MBA |
| 2011-2012 | LC-MS/MS | MBA |
| 2013-2014 | LC-MS/MS | MBA |
| 2015-2016 | LC-MS/MS | MBA |

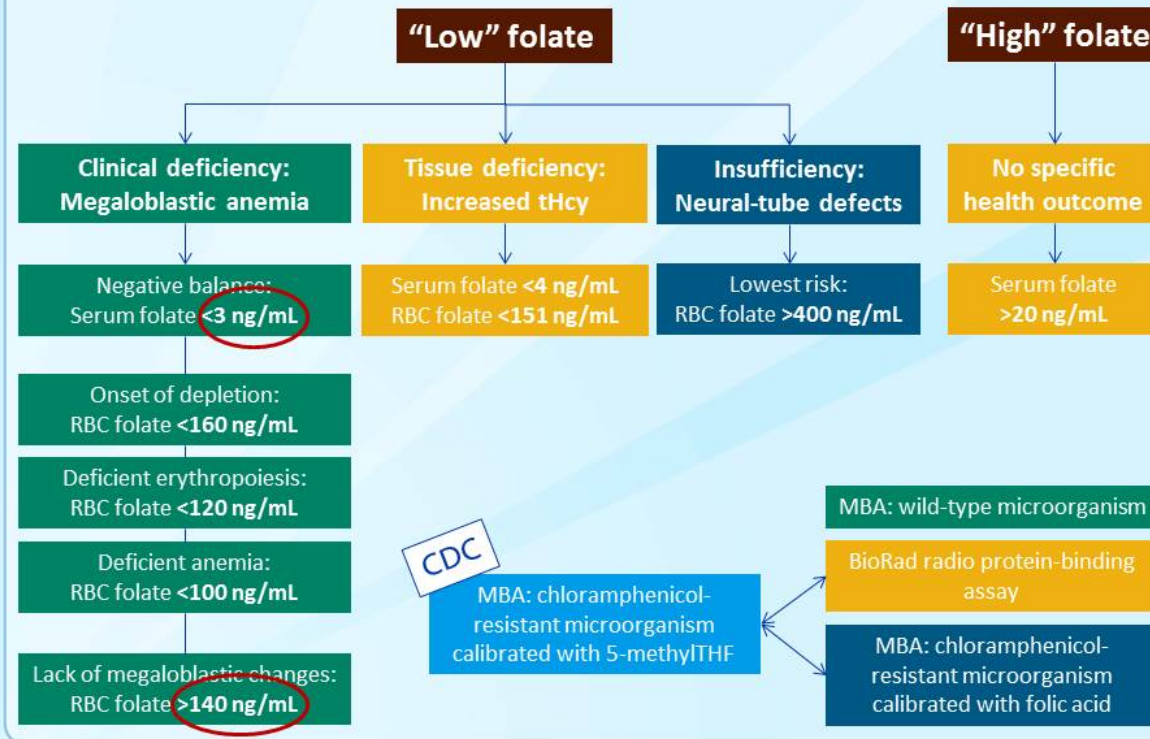
Converted to MBA-equivalent data
Pfeiffer et al. J Nutr 2012

MBA approx equivalent to LC-MS/MS
for serum folate
Fazili et al. Clin Chem 2007
Yetley et al. AJCN 2011

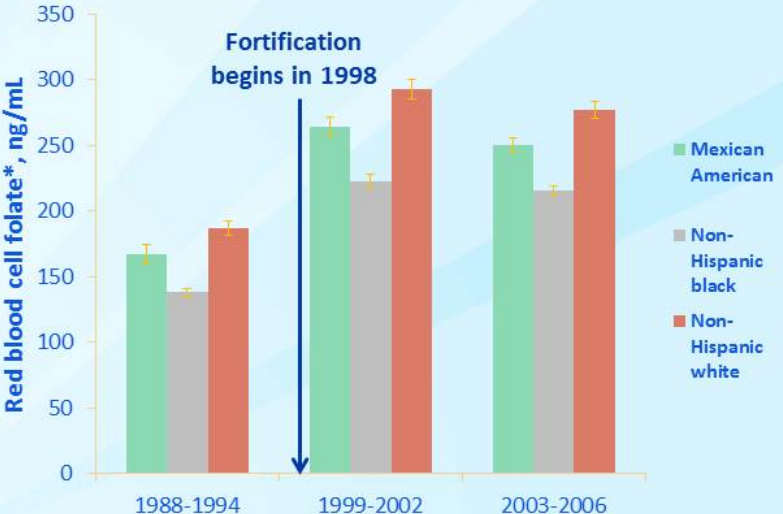
Folate methods and data in the literature

- MBA, CPBA, chromatography-based – little information on how the various methods compare at any given point in time
- MBA:
 - Limited number of labs using this assay
 - Assay results may vary depending on microorganism (antibiotic-resistant vs. wild type) and calibrator (folic acid vs. 5-methylTHF vs. 5-formylTHF)
 - Most comprehensive comparison data: *Pfeiffer et al. J Nutr 2011*
 - 2015 CDC Folate Round Robin for microbiologic assay labs
- CPBA:
 - Comparison data from a few studies, but question whether assay changed over time (*Gunter et al. Clin Chem 1996; Pfeiffer et al. Clin Chem 2001; Owen et al. Am J Clin Pathol 2003; Clifford et al. J Nutr 2005*)
- LC-MS/MS:
 - No comparison data available
 - 2015 CDC Serum Folate Round Robin for LC-MS/MS labs

Cutoff values for “abnormal” folate levels

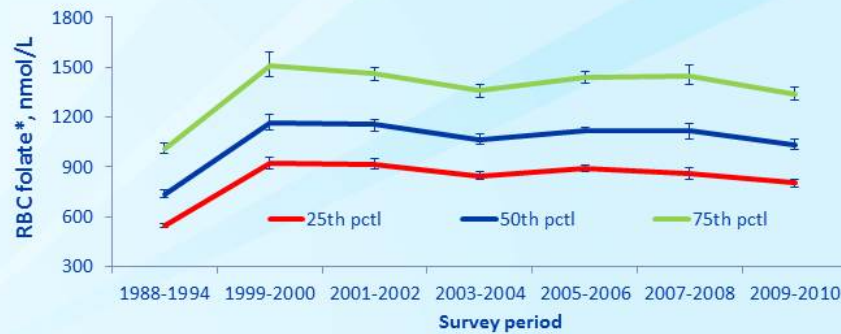
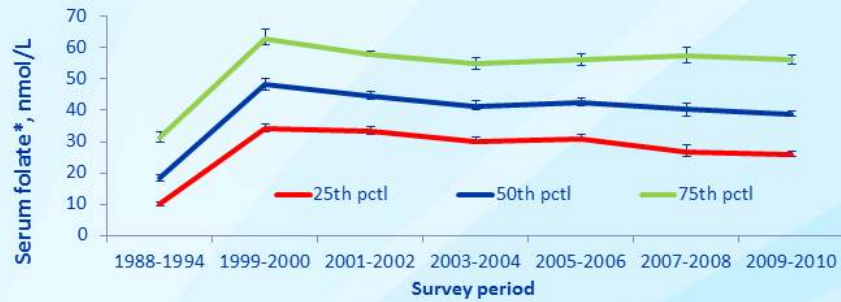


SERUM AND RBC FOLATE STATUS PRE- VS. POST-FORTIFICATION



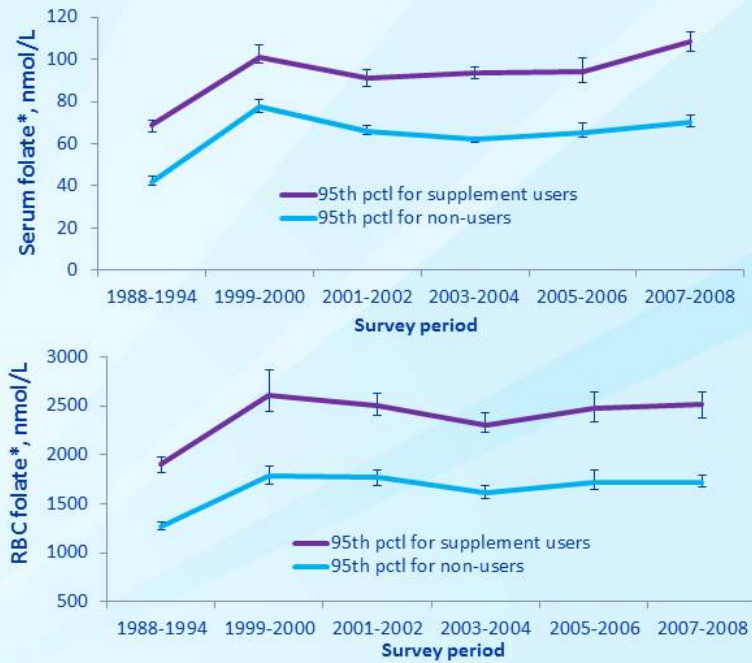
*BioRad data

Post-fortification serum and RBC folate concentrations were ~2.5x and 1.5x pre-fortification concentrations, respectively



* MBA-equivalent data

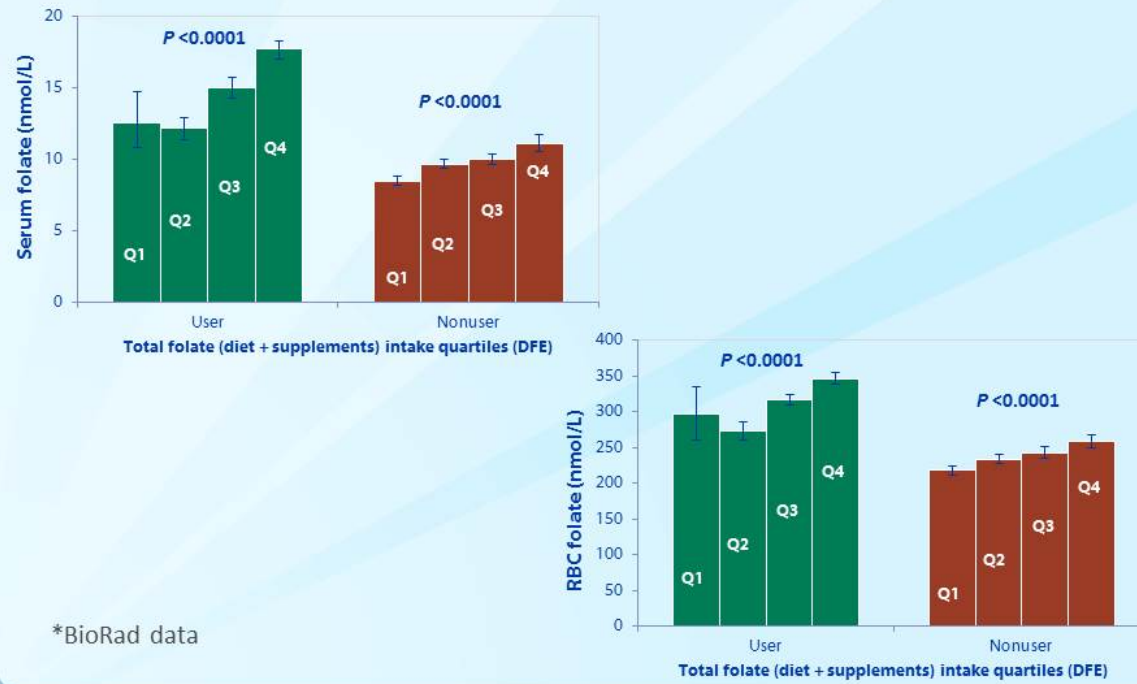
Higher serum and RBC folate 95th percentile concentrations for supplement users vs. non-users



* MBA-equivalent data

Pfeiffer et al. J Nutr 2012

Relationship between blood folate concentrations and total vitamin intake quartiles in adult supplement users and non-users NHANES 2003-2006



*BioRad data

CURRENT BLOOD FOLATE LEVELS



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Folate status and concentrations of serum folate forms in the US population: National Health and Nutrition Examination Survey 2011–2

Christine M. Pfeiffer^{1*}, Maya R. Sternberg¹, Zia Fazili¹, David A. Lacher², Mindy Zhang¹, Clifford L. Johnson², Heather C. Hamner³, Regan L. Bailey⁴, Jeanne I. Rader², Sedigheh Yamini⁵, R. J. Berry³ and Elizabeth A. Yetley¹

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U.S. Women of Childbearing Age Who Are at Possible Increased Risk of a Neural Tube Defect-Affected Pregnancy Due to Suboptimal Red Blood Cell Folate Concentrations, National Health and Nutrition Examination Survey 2007 to 2012

Sarah C. Tinker^{*1}, Heather C. Hamner², Yan Ping Qi¹, and Krista S. Crider¹

Current folate status in the US population NHANES 2007 – 2012

| Survey period | Serum folate (nmol/L) | RBC folate (nmol/L) |
|---------------|--------------------------|------------------------|
| 2007 – 2008 | 39.5 (37.7 – 41.3) | 1120 (1070 – 1160) |
| 2009 – 2010 | 38.2 (37.2 – 39.3) | 1040 (1010 – 1070) |
| 2011 – 2012 | 41.4 (40.1 – 42.9) | 1050 (1010 – 1090) |

| WHO cutoff: | 10 nmol/L | 340 nmol/L | 906 nmol/L |
|---------------|------------------------------|---------------------------|---------------------------|
| Survey period | Serum folate <13.7 nmol/L | RBC folate <624 nmol/L | RBC folate <748 nmol/L |
| 2007 – 2008 | 3.5% | 7.6% | 22.8% (women 12-49 y) |
| 2009 – 2010 | 3.9% | 9.4% | |
| 2011 – 2012 | 1.1% | 9.0% | |

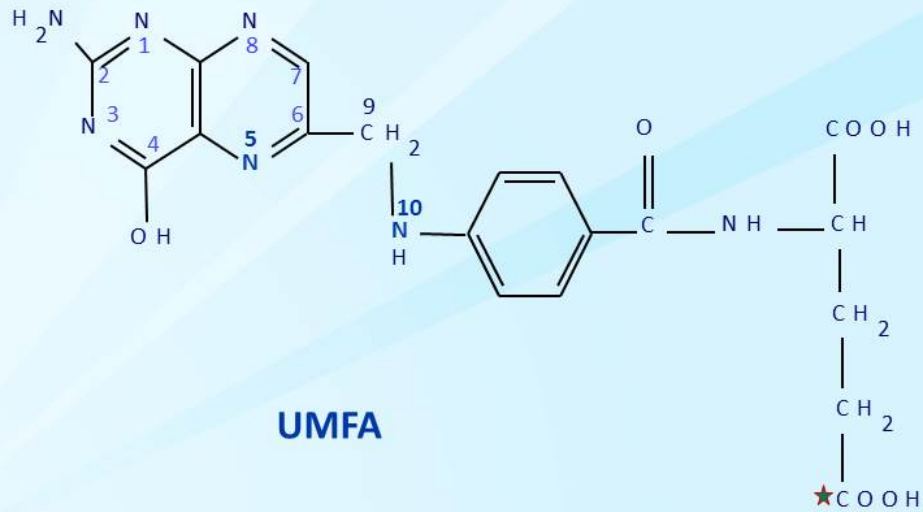
Pfeiffer et al. Br J Nutr 2015

Tinker et al. 2015

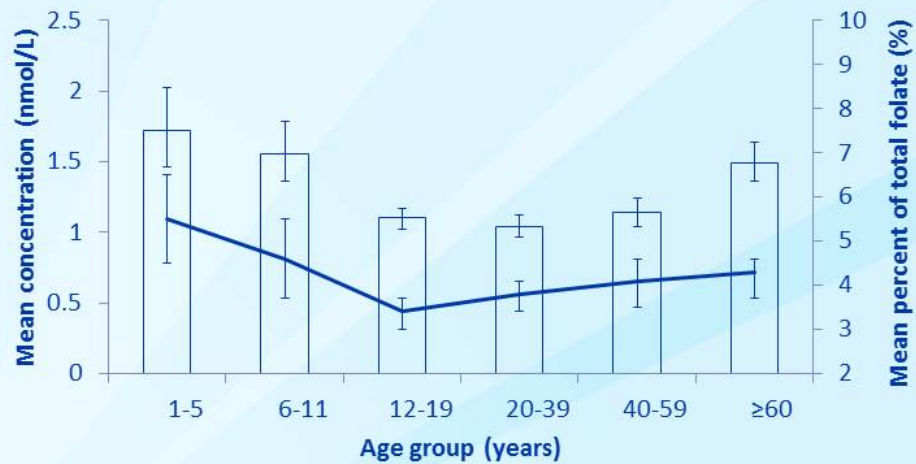
Current blood folate concentrations NHANES 2011-2012

- NHANES 2011-2012 provides the first data on serum folate forms for persons 1 y and older by demographic and selected physiologic and lifestyle variables
- Concentrations of 5-methylTHF (100%), UMFA (99.9%), MeFox (98.8%), and THF (85.2%) mostly detectable
- 5-FormylTHF (3.6%) and 5,10-methenylTHF (4.4%) rarely detected
- Contribution to total folate: 5-methylTHF (86.7%), UMFA (4%), non-methylfolate (4.7%), MeFox (4.5%)
- All biomarkers showed higher concentrations with recent folic acid-containing supplement use

SERUM UNMETABOLIZED FOLIC ACID CONCENTRATIONS




Serum UMFA contributed 4% to total folate, NHANES 2011-2012



Concentrations varied with age, sex, race-ethnicity, fasting status, eGFR, BMI, BSA, serum cotinine, alcohol intake, and folic acid supplement use

The Journal of Nutrition. First published ahead of print December 10, 2014 as doi: 10.3945/jn.114.201210.

The Journal of Nutrition
Nutritional Epidemiology 

Unmetabolized Folic Acid Is Detected in Nearly All Serum Samples from US Children, Adolescents, and Adults¹⁻⁴

Christine M Pfeiffer,^{5*} Maya R Sternberg,⁵ Zia Fazili,⁵ Elizabeth A Yetley,⁶ David A Lacher,⁷ Regan L Bailey,⁶ and Clifford L Johnson⁷

⁵National Center for Environmental Health, CDC, Atlanta, GA; ⁶Office of Dietary Supplements, NIH, Bethesda, MD; and ⁷National Center for Health Statistics, CDC, Hyattsville, MD

- Prevalence of UMFA >1 nmol/L was 33% overall and 21% in fasting (≥8 h) adults in NHANES 2007-2008
- UMFA >1 nmol/L was largely but not entirely explained by fasting status and by total folic acid intake from diet and supplements

Pfeiffer et al. J Nutr 2015

Summary

- Folate assays have not yet been standardized and results are not comparable across assays or laboratories
- Fortification has significantly increased blood folate levels in the US population
- Post-fortification prevalence of deficient blood folate levels is <10%
- Post-fortification blood folate levels have been fairly constant over a period of ~15 years
- Post-fortification detection of serum UMFA is nearly ubiquitous and concentrations >1 nmol/L are largely but not entirely explained by fasting status and by total folic acid intake from diet and supplements



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Staff of the Nutritional Biomarkers Branch
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Questions?

For more information please contact Centers for Disease Control and Prevention

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Telephone: 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348

Visit: www.cdc.gov | Contact CDC at: 1-800-CDC-INFO or www.cdc.gov/info

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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