Folate

https://open-global.kcl.ac.uk/folate/

Open access knowledge hub of Nutrition biomarkers for Global health

www.open-global.kcl.ac.uk

Date of preparation: May 2019

Contribution: Jessica Farebrother, Sarah Meadows
Dr Jessica Farebrother Dr. sc. ETH Zurich, OpeN-Global team
Sarah Meadows, OpeN-Global Expert Partner
Senior analyst and Laboratory Manager, Nutrition Biomarker Laboratory, University of Cambridge, Cambridge, UK. Website: http://www.mrc-epid.cam.ac.uk/people/sarah-meadows/

Keywords: folate – folic acid – one-carbon metabolism – homocysteine – neural tube defects – cardiovascular disease – cognitive decline – cancer – low birth weight – preterm delivery

Related nutrients/biomarkers: vitamin B12, iron, vitamin B6, vitamin B2 (riboflavin), zinc, nutrients for mitochondrial function (potassium, sodium), calcium

Complementary data analyses: prevalence of: congenital abnormalities linked to inadequate folate status e.g. neural tube defects, cardiovascular disease

Importance of folate for health

Folate is a generic term for water-soluble B vitamins that are found widely in the diet, in leafy vegetables, legumes, egg yolks, liver and some citrus fruits (1, 2). Folate is key in ensuring normal growth, development and maintenance of optimal health. Folate deficiency can have several important consequences, the most well-known being an increase in risk of neural tube defects e.g. spina bifida, due to inadequate maternal folate intakes during gestation (3-5). Public health preventive measures to ensure population-level adequate intakes include the widespread use of folic acid as a supplement and food fortificant, and 400 µg folic acid supplementation is recommended by WHO from when trying to conceive until week 12 of gestation (5). Folic acid – pteroylglutamic acid – is a synthetic vitamin seldom found in nature, however used for supplementation and fortification purposes due to its stability and low cost (4).

The function of folate is to accept, redox process and transfer one-carbon units, in the complex series of biochemical and metabolic reactions known as one-carbon metabolism (3, 4, 6). In human and mammalian cells, one-carbon metabolism occurs in the cell cytosol, mitochondria and nucleus. Folate has a major role in the body in the biosynthesis of purine and thymidine nucleotides, and the re-methylation of homocysteine to methionine. Methionine can then go on to be used in protein synthesis, or converted to S-adenosylmethionine, which is the primary methyl donor in the body (4). In this way, folate makes a critical contribution to synthesis and repair of DNA, RNA and proteins (3, 4). Body stores of folate are generally adequate for 2-3 months (3).

As folate status drops, the remethlyation of homocysteine to methionine is reduced leading to raised plasma homocysteine levels. Elevated homocysteine has been linked to several non-communicable diseases including cardiovascular disease and Alzheimer's disease.

However, though the conversion of homocysteine to methionine is dependent upon folate, it is catalysed by the MTR enzyme, which is vitamin B12 dependent. Therefore, though homocysteine is a sensitive functional biomarker of folate status, it will also be elevated with a concomitant deficiency in vitamin B12.

Risk and consequences of deficiency

Risk of folate deficiency is highest with inadequate dietary intakes; however certain physiological conditions increase requirement. These include neoplastic diseases, malabsorptive conditions (e.g. Crohn's disease and tropical sprue), the use of antifolate drugs or metabolic inhibitors (e.g. some anticonvulsants such as phenytoin, sulfasalazine and metformin) and alcoholism, which affects both folate intake and absorption.

Pregnancy is a period of increased folate requirements and risk of deficiency due to it being a period of rapid cell replication and growth, necessitating the de novo synthesis of DNA and proteins. Increased requirements continue throughout lactation (3). A deficiency during this period has been associated with several adverse gestational outcomes, including neural tube and other congenital defects (e.g. of the heart), foetal growth retardation, preterm delivery, pre-eclampsia, placental abruption, spontaneous abortion and stillbirth, low birth weight, and neonatal folate deficiency (1).

In addition to congenital deformations linked to folate deficiency during gestation, epidemiological data show an increased risk of cardiovascular disease with folate deficiency (3). This is possibly due to the high levels of circulating homocysteine, caused by an inadequate intake or metabolism of folate with vitamin B12 deficiency (3).

WHO recommendations:

Periconceptional folic acid supplementation to prevent neural tube defects

All women, from the moment they begin trying to conceive until 12 weeks of gestation, should take a folic acid supplement (400 µg folic acid daily).

Women who have had a foetus diagnosed as affected by a neural tube defect or have given birth to a baby with a neural tube defect should:

- receive information on the risk of recurrence;
- be advised on the protective effect of periconceptional folic acid supplementation;
- be offered high-dose supplementation (4000-5000 µg folic acid daily); and
- be advised to increase their food intake of folate.

See the following WHO resources for more information:

- eLENA page on Periconceptional folic acid supplementation to prevent neural tube defects: https://www.who.int/elena/titles/folate_periconceptional/en/
- Standards document: Standards for maternal and neonatal care: https://www.who.int/reproductivehealth/publications/maternal_perinatal_health/a91272/en/
- Guideline: Optimal serum and red blood cell folate concentrations in women of reproductive age for prevention of neural tube defects: https://www.who.int/nutrition/publications/guidelines/optimalserum_rbc_womenrep_tubedefects/en/

Consequences of excess

There are no reported adverse effects associated with folate intake from foods, whether from intrinsic dietary sources, or fortified foods. However, there are upper limits set on the intake of folic acid, since total folate intakes above 5000 µg/day, e.g. from additional supplement intakes, can mask vitamin B12 deficiency. If untreated, this can lead to permanent nerve damage that can be precipitated by folic acid during treatment for megaloblastic anaemia (4).

Human biomarkers of population folate intakes

Population monitoring of folate status can provide an indication of the efficacy of supplementation or fortification initiatives, alongside epidemiological prevalence data of the effects of folate deficiency such as NTDs. Folate status is most often measured in women of reproductive age and general adult populations, and data on preschool children and pregnant women are generally lacking (3). Future research efforts should also consider these life-stage groups for population surveillance.

There are several methods currently available to measure folate status, as outlined in the table below. The microbiological method is considered the gold standard of folate assessment and is suitable for use in all laboratories. It can be used with serum or whole blood.

Table: comparison of current methods in use for folate assessment (7)

Method	Advantages	Disadvantages
Microbiological assays	High sensitivity Inexpensive Low technology	Total folate only Sensitive to antibiotics and antifolates Higher imprecision than other methods 2 day assay Experienced analyst
Protein binding assays	Very high throughput Direct sample assay Minimum operator input	Total folate only Limited dynamic range Underestimation of some folate species
Chromatography	Individual species measured Chromatographic separation Large dynamic range	Experienced analyst Sample preparation required Non-specific internal standards High sample volume
LC-MS/MS	Individual species measured High sensitivity High Specificity Matched Internal standards Large dynamic range	Experienced analyst Expensive instrumentation Sample preparation required Chromatographic separation limited

Serum folate is a marker of acute folate status and red blood cell (RBC) folate one of chronic status, but several repeated low measurements of serum folate can also indicate deficiency.

The UK and USA use LC-MS/MS for population monitoring specifically to be able to distinguish between folic acid and other endogenous folates due to monitoring fortification and any adverse side effects.

Methods

- *Total folate*
    - Microbiologic assay for use with serum or whole blood: https://www.cdc.gov/nchs/data/nhanes/nhanes_11_12/FOLATE_G_met_RBC.pdf
- *Folate forms*
    - For use with serum or whole blood, using liquid chromatography tandem mass spectrometry (LC-MS): https://www.cdc.gov/nchs/data/nhanes/nhanes_11_12/FOLFMS_G_met_FOLATE_FORMS.pdf
- *Folate with B12*
    - Using a kit with serum or whole blood: https://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/l06_c_met_folates%20B12.pdf

Sample stability

Serum folate: Serum folate is stable in the refrigerator for up to 1 week or for several years if stored frozen at -70°C. Serum folates are stable through 3 freeze thaw cycles with minimal exposure to ambient temperature but deteriorate with further freeze thaw cycles. Serum folates show up to a 10% loss when exposed to ambient temperature for 5 hours (8).

Red cell folate: The short term stability of whole blood total folate in unfrozen haemolysates is worse than that for whole blood but haemolysates are stable for up to 4 days if refrigerated and for several years if stored frozen at -70°C. Whole blood folate without tetrahydrofolate shows good stability with up to 3 freeze thaw cycles but a 20% loss after 2 freeze thaw cycles with samples containing tetrahydrofolate (8).

Laboratory accreditation

The US CDC **VITAL-EQA** program helps labs maintain and improve the quality of their measurements for biochemical indicators, including serum folate: https://www.cdc.gov/labstandards/vitaleqa_about.html

If you are interested in participating in the program, or would simply like further information, write to vitaminalab@cdc.gov

For more details on other laboratory accreditation, validation or proficiency testing schemes, please see the OpeN-Global page on Laboratory accreditation: https://open-global.kcl.ac.uk/accreditation/

Folate-specific information: UK NEQAS have a heamatinics scheme which includes total folate in serum and red cells: https://ukneqas.org.uk

Technical assistance

For questions on methods of folate assessment or for technical assistance, please contact the OpeN-Global team at https://open-global.kcl.ac.uk/contact/ or the Nutrition Biomarker Laboratory, University of Cambridge, Cambridge, UK

nbl@mrc-epid.cam.ac.uk

Useful links and further reading

Biomarkers of Nutrition for Development (BOND) review for folate:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4478945/

Resources from the US CDC:

- CDC general resource on Folate: https://www.cdc.gov/ncbddd/folicacid/index.html
- Framework for laboratory harmonization of folate measurements in low-and middle-income countries and regions: https://nyaspubs.onlinelibrary.wiley.com/doi/10.1111/nyas.13532
- VITAL EQA Program for laboratory quality assurance:
https://www.cdc.gov/labstandards/vitaleqa_about.html
- Resource providing links to scientific articles on folate and birth defects:
https://www.cdc.gov/ncbddd/birthdefectscount/obf-related-articles.html

Resources from the WHO:

- WHO Technical consultation on folate and vitamin B12 deficiencies*:*
https://www.who.int/nutrition/publications/micronutrients/FNBvol29N2supjun08.pdf
- eLENA page on Periconceptional folic acid supplementation to prevent neural tube defects:
https://www.who.int/elena/titles/folate_periconceptional/en/
- Standards document: Standards for maternal and neonatal care:
https://www.who.int/reproductivehealth/publications/maternal_perinatal_health/a91272/en/

- Guideline: Optimal serum and red blood cell folate concentrations in women of reproductive age for prevention of neural tube defects: https://www.who.int/nutrition/publications/guidelines/optimalserum_rbc_womenrep_tubedefects/en/

- Publication: Conclusions of a WHO Technical Consultation on folate and vitamin B12 deficiencies: https://www.who.int/nutrition/publications/micronutrients/FNBvol29N2supjun08.pdf

- Watson, J., Lee, M. & Garcia-Casal, M.N. Consequences of Inadequate Intakes of Vitamin A, Vitamin B12, Vitamin D, Calcium, Iron, and Folate in Older Persons Curr Geri Rep (2018) 7: 103. : https://link.springer.com/article/10.1007%2Fs13670-018-0241-5

Resources from the UK National Diet and Nutrition Survey:

- NDNS: blood folate supplementary report. Data on blood folate concentrations published as part of the National Diet and Nutrition Survey (NDNS): https://www.gov.uk/government/statistics/national-diet-and-nutrition-survey-supplementary-report-blood-folate

The Folate Task Team at Nutrition International

The Folate Task Team at Nutrition International, is a global nutrition initiative for the control of folate insufficiency and folic acid responsive neural tube defects. Through Nutrition Technical Assistance Mechanism(NTEAM)'s Folate Task Team project, Nutrition International is helping to lay the groundwork for implementing a global strategy for the control of folate insufficiency and prevention of related neural tube defects (NTDs).

See the link below to see all details and access past Webinar training recordings and more information from the Folate Task Team.

https://www.nutritionintl.org/what-we-do/nteam/folate-task-team/

Other resources:

Micronutrient forum technical consultation on improving NTD status of LMIC countries: https://micronutrientforum.org/content/user_files/2017/10/2017-07FolateTechnicalConsultation-FinalReport.pdf

EU EFSA Report Folic Acid: An update on scientific developments: https://www.efsa.europa.eu/en/supporting/pub/en-2

NIH Health Professional Factsheet: Folate: https://ods.od.nih.gov/factsheets/Folate-HealthProfessional/

US National Academies Press IOM Dietary reference intakes for thiamine, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline, 2000: https://www.ncbi.nlm.nih.gov/books/NBK114310/

Novaković R, Geelen A, Ristić-Medić D, Nikolić M, Souverein O, W, McNulty H, Duffy M, Hoey L, Dullemeijer C, Renkema J, M, S, Gurinović M, Glibetić M, de Groot L, C, P, G, M, van't Veer P: Systematic Review of Observational Studies with Dose-Response Meta-Analysis between Folate Intake and Status Biomarkers in Adults and the Elderly. Ann Nutr Metab 2018;73:30-43: https://www.karger.com/Article/FullText/490003

SobczyńskaMaleforaA, HarringtonDJ. Laboratory assessment of folate (vitamin B_9) status. Journal of Clinical Pathology 2018;71:949-956: https://jcp.bmj.com/content/71/11/949

References

1. World Health Organization 2012 Serum and red blood cell folate concentrations for assessing folate status in populations. . In: System. VaMNI, (ed). World Health Organization, Geneva, Switzerland.

2. Kamen B 1997 Folate and antifolate pharmacology. Seminars in oncology **24**:S18-30-s18-39.

3. Bailey LB, Stover PJ, McNulty H, Fenech MF, Gregory JF, 3rd, Mills JL, Pfeiffer CM, Fazili Z, Zhang M, Ueland PM, Molloy AM, Caudill MA, Shane B, Berry RJ, Bailey RL, Hausman DB, Raghavan R, Raiten DJ 2015 Biomarkers of Nutrition for Development-Folate Review. The Journal of nutrition **145**:1636S-1680S.

4. Chan YM, Bailey R, O'Connor DL 2013 Folate. Adv Nutr **4**:123-125.

5. World Health Organization. Periconceptional folic acid supplementation to prevent neural tube defects. http://www.who.int/elena/titles/folate_periconceptional/en/. (Last accessed on August 28, 2018).

6. US Institute of Medicine 1998 Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Chapter 8, Folate. The National Academies Press, Washington, DC.

7. Meadows S 2016 Measurement of serum folate vitamers by LC-MS/MS. Clinical Laboratory International, **40**:18-20.

8. Bailey LE 2017 Folate in Health and Disease (2nd Edition). CRC Press, Boca Raton.