Evidence for Fortification of Wheat Flour with Folic Acid and Vitamin B12

Photo copyright: David Snyder / CDC Foundation

Compiled by Becky Tsang
Food Fortification Initiative
Technical Officer in Asia

Food Fortification Initiative
Enhancing Grains for Healthier Lives

Contents

Executive summary 3

Folic acid in fortified wheat flour to prevent neural tube defects 4

Folic acid in fortified wheat flour to improve folate status 4

Folic acid in fortified wheat flour to reduce anemia and folate-deficiency anemia 6

Folic acid in fortified wheat flour to prevent heart disease and stroke 7

Vitamin B12 in fortified wheat flour to improve vitamin B12 status 7

Folate and vitamin B12 concentrations and their relationship to cognitive impairment and biomarkers of vitamin B12 metabolism 7

Folic acid masking of vitamin B12 deficiency 8

References: 9

In 2009, the World Health Organization (WHO) published international guidelines for the fortification of wheat and maize flour (1). Folic acid and vitamin B12 were included in WHO's guidelines based on evidence that neural tube defects were reduced in several countries after fortification of wheat flour with folic acid, and the feasibility of improving vitamin B12 intake through fortification.

The purpose of this summary is to compile evidence from efficacy trials (i.e. randomized controlled trials) and effectiveness studies (i.e. implementation under real-life programmatic conditions) on the impact of wheat flour fortified with folic acid and B12 on several outcomes, including: improved folate status, folate-deficiency anemia reduction, neural tube defect prevention, heart disease and stroke prevention, improved vitamin B12 status, declining cognition function, and masking of vitamin B12 deficiency.

Folic acid *is a compound added to food during fortification. It is also used in supplements.* ***Folate*** *is measured as a marker in biological samples taken from humans. When people consume folic acid, their blood folate level increases.*

Wheat flour is the target of this summary because it is the food vehicle most commonly fortified with folic acid, but a fortification vehicle should be chosen based on a population's dietary patterns. Wheat flour may not be suitable for fortification if it is not consumed in adequate quantities (>= 75 g/person/day) or is not industrially milled.

This peer-reviewed summary was written by the Food Fortification Initiative. For more information, e-mail info@ffinetwork.org or see: www.FFInetwork.org

How to cite this document: Food Fortification Initiative (FFI). Evidence for Fortification of Wheat Flour with Folic Acid and Vitamin B12. Atlanta, USA: FFI, 2017. Available from www.FFInetwork.org Accessed on [date].

Executive summary

Folic acid in fortified wheat flour to prevent neural tube defects

There is strong evidence that wheat flour fortified with folic acid prevents neural tube defects, which are birth defects of the brain and spine. Although the available data are cross-sectional, pre-post fortification analyses, the study outcome (reduced neural tube defect prevalence) is consistent across studies and feasible considering the strong evidence from randomized controlled trials and cohort studies of folic acid's prevention of neural tube defects.

Folic acid in fortified wheat flour to improve folate status

There is strong evidence globally that wheat flour fortified with folic acid has improved blood folate concentrations and reduced the prevalence of folate deficiency. Although much of this data are cross-sectional, the study outcomes are consistent across studies. The outcomes are also feasible considering supporting evidence from limited efficacy trials of fortified wheat flour products and folic acid supplements to improve blood folate concentrations.

Folic acid in fortified wheat flour to reduce anemia and folate-deficiency anemia

There is limited evidence that wheat flour fortified with folic acid reduces anemia and folate-deficiency anemia. The evidence is cross-sectional, but the outcomes are feasible and consistent with evidence for the improvement of blood folate concentrations with fortified wheat flour.

Folic acid in fortified wheat flour to prevent heart disease and stroke

There is strong, but limited, evidence that folic acid supplements (combined with hypertension medication) prevent primary stroke. There is no evidence that folic acid in fortified wheat flour prevents heart disease or stroke.

Vitamin B12 in fortified wheat flour to improve vitamin B12 status

There is limited but consistent evidence of the impact of vitamin B12 in fortified wheat flour to improve vitamin B12 status.

Folate and vitamin B12 concentrations and their relationship to cognitive impairment and biomarkers of vitamin B12 metabolism

There is weak evidence that high folate concentrations are harmful in individuals with low vitamin B12 status. The available data are cross-sectional, the study outcomes (cognitive impairment, anemia, high methylmalonic acid and homocysteine concentrations) are inconsistent across studies, and there is no supporting evidence from randomized controlled trials or cohort studies. It is plausible that confounding (malabsorption) or reverse causality are feasible explanations for the high folate/low vitamin B12 findings. The high folate concentrations found in these studies are also most likely due to high supplement use in elderly populations, not folic acid contributed by fortified foods.

Folic acid masking of vitamin B12 deficiency

There is weak evidence for the masking of vitamin B12 deficiency with folic acid supplements or folic acid fortification in the context of modern medicine. Historical evidence is only available as case reports, conducted prior to the awareness of megaloblastic anemia attributed to vitamin B12 deficiency. There are limited studies on the prevalence of "masked" vitamin B12 deficiency (i.e. without anemia) in the context of mandatory folic acid fortification, but the results are consistent and found no increased prevalence of vitamin B12 deficiency without anemia after fortification in the United States.

Folic acid in fortified wheat flour to prevent neural tube defects

<u>Efficacy trials</u>

There are no efficacy studies assessing the impact of fortified wheat flour on neural tube defects. This is likely because the sample sizes required for assessing neural tube defects as an outcome would be high in a randomized controlled trial.

Baby with spina bifida, one type of neural tube defect. Photo from the International Federation for Spina Bifida and Hydrocephalus.

However, the evidence for folic acid's role in preventing neural tube defects is well established in clinical trials and cohort studies, which conclusively showed that folic acid supplementation prevents 50-70% of neural tube defects (2-4). The evidence for the prevention of neural tube defects using folic acid was summarized by a Cochrane Collaboration Review in 2012, concluding that "…folic acid supplementation prevents the first and second time occurrence of neural tube defects…" (5). Although folic acid supplementation is efficacious in preventing neural tube defects, because folic acid supplementation must achieve high coverage for adequate prevention (6), its effectiveness as a population-wide intervention is poor. Assessments of educational campaigns have shown that supplementation compliance among women in the critical peri-conceptional period is low (7,8). Countries with national folic acid supplementation recommendations have not reported improvements in neural tube defect prevalence (9).

<u>Effectiveness studies</u>

Multiple and consistent studies from pre-fortification to the post-fortification periods in countries show reductions in neural tube defect prevalence after wheat flour was fortified with folic acid.

In 1996, Oman became the first country to reach national-scale fortification of wheat flour with folic acid to prevent neural tube defects (10). In July 2017, 81 countries included folic acid in mandatory standards for national wheat flour fortification programs (11). The global success was summarized in a meta-analysis which found that across eight studies, the estimated reduction in incidence of neural tube defects was 46% (6).

Another systematic review of 27 studies assessing the impact of fortifying flour with folic acid found that in all countries with available data, "fortification of flour with folic acid has had a major impact" (12).

Folic acid in fortified wheat flour to improve folate status

<u>Efficacy trials</u>

Three efficacy trials assessing the impact of fortified wheat products (flour, bread, breakfast cereals) showed improved blood folate concentrations. Two of these studies also assessed impact on vitamin B12 concentrations.

Winkels et al. (13) studied 142 Dutch participants over a 12-week period who consumed an average of 4.6 slices of bread daily fortified to provide 138 µg of folic acid and 9.6 µg of vitamin B12. Compared with participants who consumed non-fortified bread, fortified bread intake led to significantly increased serum folate concentrations by 46% (from 15.7 to 20.6 nmol/L), RBC folate concentrations by 22% (from 683 to 790 nmol/L), serum vitamin B12 concentrations by 49% (from 223 to 331 pmol/L), and decreased homocysteine concentrations by 13% (10.5 to 8.9 µmol/L).

In the United States, Tucker et al. (14) co-conducted a study in 179 individuals with a daily intake of 1 cup of fortified breakfast cereals over 12 weeks (with 440 µg of folic acid[1], 4.8 µg of vitamin B12, and 1.8 mg of vitamin B6). Fortification reduced folate deficiency (plasma folate <11 nmol/L) in the study population from 2% to 0%, vitamin B12 deficiency (<185 pmol/L) from 9% to 3%, and vitamin B6 deficiency (<20 nmol/L) from 6% to 2%. Prevalence of high homocysteine concentrations (>10.4 µmol/L for women; >11.4 µmol/L for men) was also reduced from 6.4% to 1.6%. In contrast, in control subjects, the prevalence of vitamin B deficiencies or high homocysteine either stayed constant or increased.

In China, Huo et al. (16) demonstrated that women receiving wheat flour fortified with vitamin A, iron, zinc, and the B vitamins folic acid, thiamin, and niacin experienced a significant improvement in serum folate concentrations, with an increase from 20.3 nmol/L at baseline to 21.5 nmol/L[2] at the 36 month end-line period. In addition, the effects were also positive for serum retinol, serum zinc, and serum iron, but not free erythrocyte protoporphyrin.

Effectiveness studies

Twelve effectiveness studies assessing the impact of fortified wheat products (flour, bread, breakfast cereals) were found to improve blood folate concentrations.

Using a prospective, non-experimental design (i.e. no control group), Varea et al. (17) assessed the impact of a fortified food basket (fortified wheat flour with 2.2 mg/kg of folic acid, fortified maize with 1 mg/kg, fortified soup with 49% of average daily requirement of folic acid[3]) on micronutrient status in lactating Argentine mothers. The maize and wheat flours were intended for general household use while the fortified soup was targeted specifically to lactating women. Folate deficiency (<6.8 nmol/L) in mothers receiving this fortified food basket for 12 months declined from 50.3% to 3.4%, with an increase in mean serum folate from 6.7 nmol/L to 18.4 nmol/L[4]. Additionally, the study assessed the impact of the fortified food basket on ferritin concentrations, iron deficiency, zinc concentrations, zinc deficiency, retinol concentrations, vitamin A deficiency and marginal vitamin A deficiency. Except for retinol and prevalence of marginal vitamin A deficiency, the impact of the fortified food basket on the rest of the biomarkers and prevalence of deficiency were not significant.

Mandatory fortification of wheat flour in Chile was expected to provide an additional 400 µg of folic acid daily. After implementation, Hertrampf et al. (18) measured folic acid content of bread at retail bakeries, assessed dietary intake of fortified bread, and blood folate and vitamin B12 concentrations in women of childbearing age at participating outpatient clinics (wheat flour in Chile does not include vitamin B12). None of the women reported consuming other folic acid fortified foods or supplements. Post-fortification, the average daily folic acid intake was 427 µg (95% CI: 409-445 µg). Ten months post-fortification, both serum and red blood cell (RBC) folate increased significantly (serum folate: from 9.7 nmol/L to 37.2 nmol/L; RBC folate: from 290 nmol/L to 707 nmol/L), and folate deficiency was eliminated in the study population. As expected, there were no changes to vitamin B12 status. Similarly, after a one-year mandatory wheat flour fortification program in Cameroon,

---

[1] The Institute of Medicine (IOM) (15) recommends intake for women of reproductive age is 400 µg of folic acid daily; the upper limit as defined by IOM is 1,000 µg of folic acid daily.
[2] Originally reported as ng/mL, values are reported here as nmol/L, using 2.266 nmol/L = 1 ng/mL, to maintain unit consistency with other publications. The unconverted values are 8.96 ng/mL at baseline and 9.5 ng/mL at end-line.
[3] Per kg, the wheat flour was co-fortified with 30 mg of iron as ferrous sulfate, 6.3 mg thiamine, 13 mg niacin, and 1.3 mg riboflavin; per kg the maize flour was co-fortified with 1,500 µg RE vitamin A, 8 mg thiamine, 8 mg riboflavin, 100 mg niacin, 40 mg of iron as ferrous sulfate, 30 mg of zinc as zinc sulfate. The soup was co-fortified to provide 18.4% calcium, 66.6% iron, 33.3% zinc, 20.7% vitamin A, 60% vitamin D, 16.6% vitamin C, 11.2% magnesium, 28.5% selenium, 50% thiamine, 43.6% riboflavin, 40% niacin, 45% vitamin B6, and 32% vitamin B12.
[4] 2.96 ng/mL to 8.12 ng/mL.

women's plasma folate concentration significantly improved from 15 nmol/L in 2009 (baseline) to 47 nmol/L in 2012 (P<0.0001) (19).

Increased blood folate concentrations after fortification of wheat flour with folic acid was documented in several countries, using both national level nutrition surveys, and subnational studies in subgroup populations, including Azerbaijan (20), Canada (21, 22), Chile (23), Costa Rica (23, 24), Fiji (25), Guatemala (23), Iran (26), Kazakhstan (9), Mexico (23), Mongolia (20), South Africa (27), Tajikistan (20), the United States (28), and Uzbekistan (20). Pre-fortification, prevalence of folate deficiency ranged from 1.7-42% in Latin America (23), 24% (serum folate <10 nmol/L) and 3.5% (RBC folate <340 nmol/L) in the United States, and 14.3% (serum folate <6.7 nmol/L) in Iran. In all pre-post fortification assessments, prevalence of folate deficiency fell to below 3% except in Central Asian countries (except Kyrgyzstan) and Mongolia. The Central Asian countries (except Kyrgyzstan) and Mongolia saw significant decreases in folate deficiency but prevalence still exceeded 10% in all countries four years after the pilot implementation project (20).

Folic acid in fortified wheat flour to reduce anemia and folate-deficiency anemia

Efficacy trials

No efficacy trials were found on the impact of fortified wheat flour in reducing anemia or folate-deficiency anemia. There is sufficient evidence that folic acid in fortified foods improves blood folate concentrations, which will reduce folate-deficiency anemia.

Effectiveness studies

The US fortification program delivers an estimated additional 100-200 μg of folic acid daily to women of childbearing age (29). Odewole et al. (30) assessed folate-deficiency anemia (serum folate <6.6 nmol/L and hemoglobin <12 g/dL (women) or <13 g/dL (men)) in a population of adults over 45 years of age (REGARDS study) in the post-fortification period. Prior to fortification, in the 1991-1994 NHANES[5] period, folate-deficiency anemia accounted for ~30% of anemia in older adults (31). Folate-deficiency alone accounted for 6.4% of anemia, folate and B12 deficiencies combined accounted for 2% of anemia, and the combination of iron, folate, or B12 deficiencies accounted for another 3% of anemia (31). Post-fortification, Odewole et al. (30) found folate-deficiency anemia was <0.1%, or 1 individual out of 1546 with blood measurements.

Ganji et al. (32) assessed NHANES data pre- and post-fortification, and found that after the inclusion of folic acid in the fortification program, hemoglobin levels significantly increased from 13.3 g/dL to 13.6 g/dL in women (P<0.0001) and from 15.1 g/dL to 15.4 g/dL in men (P<0.0001). While the prevalence of anemia in men did not significantly improve (3.3% to 2.9%), the prevalence of anemia (<12 g/dL) in women significantly reduced in the post-fortification period compared to pre-fortification (10.4% to 7.5%, a 28% decrease), and the decline in anemia was consistent across all ethnic/racial groups, poverty: income ratio groups, age ranges, and regardless of whether women used vitamin or mineral supplements.

[5] US NHANES: National Health and Nutrition Examination Survey, a nationally representative survey of adults and children in the United States. http://www.cdc.gov/nchs/nhanes.htm

Folic acid in fortified wheat flour to prevent heart disease and stroke

Efficacy trials

No efficacy trials of the impact of fortified wheat flour to prevent heart disease were found. A randomized controlled trial of fortified wheat flour on a long-term outcome such as heart disease would be extremely resource-intensive.

Although the focus of this brief is fortification, there are efficacy trials of folic acid supplementation for the primary prevention of cardiovascular disease. A review conducted for the United States Preventive Services Task Force (33) found that for the few studies assessing folic acid, there is no effect on cardiovascular disease. In 2015, Huo et al. (34) completed a randomized controlled trial in China and found that for the prevention of primary stroke in adults, daily folic acid (0.8 mg) combined with hypertension medication enalapril (10 mg) reduced the risk of stroke (Hazard Ratio (HR) =0.79; 95% CI, 0.68-0.93) compared to enalapril alone. Yang et al. (35), in another systematic review, found no relationship with folic acid supplements on cardiovascular disease but a potential benefit for stroke prevention (Risk Ratio (RR) =0.93, 95% CI, 0.86-1.00).

When interpreting these supplementation efficacy trials in the context of fortification, it should be noted that populations will consume folic acid in fortified foods at much lower levels than with supplements.

Effectiveness studies

No effectiveness studies were found of the impact of fortified wheat flour in preventing heart disease and stroke.

Vitamin B12 in fortified wheat flour to improve vitamin B12 status

Efficacy trials

Winkels et al. (13) and Tucker et al.'s (14) studies on the impact of fortified bread and breakfast cereals with vitamin B12 to improve vitamin B12 status are discussed in the "Folic acid in fortified wheat flour to improve folate status" section above.

Effectiveness studies

Engle-Stone et al. (19) conducted a pre- and post-fortification study in Cameroon to determine the effect of wheat flour fortification on micronutrient levels. The study found that after a one-year mandatory wheat flour fortification program, plasma vitamin B-12 concentrations in women significantly increased from 461 pmol/L in 2009 (baseline) to 671 pmol/L in 2012 ($P<0.0001$).

Folate and vitamin B12 concentrations and their relationship to cognitive impairment and biomarkers of vitamin B12 metabolism

Efficacy trials

No efficacy trials of folic acid and vitamin B12 interactions were found. Ethical considerations do not allow for randomized controlled trials to be conducted in individuals with B12 deficiency.

Effectiveness studies

Morris et al. (36), and Miller et al. (37), using two cross-sectional studies from the United States (NHANES and SALSA), showed an association between high methylmalonic acid (MMA) concentrations (>210 nmol/L in Morris et al.) and high homocysteine concentrations among older adults with low vitamin B12 status (plasma or serum

<148 pmol/L) and high folate status (serum folate >59 nmol/L in Morris, et al.; plasma folate >45.3 nmol/L in Miller, et al.). While Morris et al. also found a relationship with cognitive impairment and anemia, Miller et al. did not assess anemia. Castillo-Lancelotti et al. (38) in Chile, and Moore et al. (39) in Australia found an association between high blood folate status and low vitamin B12 status with cognitive impairment only (they did not measure other vitamin B12 indicators).

However, two studies have not replicated the results: Clarke et al. (40), using data from two cross-sectional studies of older adults in the UK, and Mills et al. (41), with cross-sectional data from Irish university students, have shown the opposite: no relationship between indicators of poor vitamin B12 metabolism, cognitive impairment, or anemia in individuals with low vitamin B12 status and high blood folate status. National level data from Canada, reported by McFarlane et al. (42), also did not find that individuals with high RBC folate status (>1090 nmol/L) were more likely to be vitamin B12 deficient (≤ 148 pmol/L); in contrast, high RBC folate status was associated with adequate vitamin B12 status and normal homocysteine status (<13 µmol/L).

The major weakness across these studies is the use of cross-sectional study designs, which do not allow for conclusions of causality in the relationship between high folate and low vitamin B12 status and these outcomes of interest. Critiques of these studies (43, 44) have suggested the following two alternate explanations for the relationship between cognitive impairment and low B12 status and high folate status:

- Reverse causality, as advanced vitamin B12 deficiency typically raises folate concentrations 20-30% (43).
- Malabsorption, as the intrinsic factor required to absorb vitamin B12 decreases as adults age.

Berry et al. (44) found that in the US NHANES population (the population in Morris et al.'s study), 92% of the older US adults in the highest serum folate quintile were consuming folic acid supplements; 97% of those supplements contained vitamin B12 as well. Low vitamin B12 status and high folate status in a population consuming multivitamins with both folic acid and vitamin B12 suggests that the associated adverse effects could be attributed to low vitamin B12 status due to malabsorption (44). Finally, it should be noted that the high folate concentrations discussed in these cross-sectional studies are likely due to supplements containing folic acid, not fortified foods with folic acid. This has been confirmed in the NHANES population (45).

Folic acid masking of vitamin B12 deficiency

Folic acid masking of vitamin B12 deficiency was a medical concern when hematological blood smears were the primary diagnostic tool available to measure vitamin B12 deficiency. In modern medicine it is possible to diagnose vitamin B12 deficiency with greater specificity than blood smears; as such the potential for folic acid to mask vitamin B12 deficiency is no longer a concern. However, due to concerns regarding potential vitamin B12 deficiency masking, an overview of the evidence is provided below.

Efficacy trials

Ethical considerations do not allow for randomized controlled trials to be conducted in individuals with vitamin B12 deficiency.

Case reports

The first case reports of vitamin B12 "masking" occurred in the 1940s as a result of high folic acid doses being used to treat megaloblastic anemia that was in fact due to vitamin B12 deficiency (which was not known) (29). Treating these individuals with folic acid resolved the megaloblastic anemia but did not address the neurological damage due to vitamin B12 deficiency. Today, due to advances in diagnoses and treatment of vitamin B12 deficiency, "masking" of vitamin B12 deficiency would only be a concern in medical settings where blood smears are the only hematological lab assessment, and high folic acid (>5 mg/day) doses are being provided. The

Government of New Zealand's scientific evaluation of concerns of folic acid fortification in 2012 addressed masking in modern medicine: "This theoretical risk is considered negligible in current medical practice. Laboratory methods that directly test for the level of vitamin B12 in the blood are now widely available" (46).

Effectiveness studies

Qi et al. (47) and Mills et al. (48), using cross-sectional data from the pre- and post-folic acid fortification periods in the United States, both found no increase of vitamin B12 deficiency (<148 pmol/L) without anemia in the post-fortification period, suggesting that vitamin B12 deficiency has not been "masked" by resolved anemia due to increased access to foods fortified with folic acid in the United States.

References:

1. World Health Organization, Food and Agriculture Organization of the United Nations, United Nations Children's Fund, Global Alliance for Improved Nutrition, the Micronutrient Initiative, and Flour Fortification Initiative. Recommendations on wheat and maize flour fortification. Meeting report: interim consensus statement. Geneva: World Health Organization. 2009.
2. Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. N Engl J Med. 1992;327(26):1832-5.
3. Berry RJ, Li Z, Erickson JD, Li S, Moore CA, Wang H, et al. Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. N Engl J Med. 1999;341(20):1485-90.
4. MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. Lancet. 1991;338(8760):131-7.
5. De-Regil LM, Fernandez-Gaxiola AC, Dowswell T, Peña-Rosas JP. Effects and safety of periconceptional folate supplementation for preventing birth defects. Cochrane Database Syst Rev. 2010(10):CD007950.
6. Blencowe H, Cousens S, Modell B, Lawn J. Folic acid to reduce neonatal mortality from neural tube disorders. Int J Epidemiol. 2010;39 Suppl 1:i110-21.
7. Ray JG, Singh G, Burrows RF. Evidence for suboptimal use of periconceptional folic acid supplements globally. BJOG. 2004;111(5):399-408.
8. Ward M, Hutton J, Mc Donnell R, Bachir N, Scallan E, O'Leary M, et al. Folic acid supplements to prevent neural tube defects: trends in East of Ireland 1996-2002. Ir Med J. 2004;97(9):274-6.
9. Botto LD, Lisi A, Robert-Gnansia E, Erickson JD, Vollset SE, Mastroiacovo P, et al. International retrospective cohort study of neural tube defects in relation to folic acid recommendations: are the recommendations working? BMJ. 2005;330(7491):571.
10. Zimmerman S. Fifteen years of fortifying with folic acid: birth defects are reduced and healthcare expenses are averted. Sight and Life. 2011;25:54-59.
11. Food Fortification Initiative (FFI). Global Progress. Atlanta, USA: FFI; 2017. Available from: http://ffinetwork.org/global_progress/index.php
12. Castillo-Lancellotti C, Tur JA, Uauy R. Impact of folic acid fortification of flour on neural tube defects: a systematic review. Public Health Nutr. 2013;16(5):901-11.
13. Winkels RM, Brouwer IA, Clarke R, Katan MB, Verhoef P. Bread cofortified with folic acid and vitamin B-12 improves the folate and vitamin B-12 status of healthy older people: a randomized controlled trial. Am J Clin Nutr. 2008;88(2):348-55.
14. Tucker KL, Olson B, Bakun P, Dallal GE, Selhub J, Rosenberg IH. Breakfast cereal fortified with folic acid, vitamin B-6, and vitamin B-12 increases vitamin concentrations and reduces homocysteine concentrations: a randomized trial. Am J Clin Nutr. 2004;79(5):805-11.
15. Institute of Medicine (US). Dietary Reference Intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington (DC): National Academies Press (US); 1998. Available from: https://www.ncbi.nlm.nih.gov/books/NBK114318/.

16. Huo J, Sun J, Huang J, Li W, Wang L, Selenje L, et al. Effectiveness of fortified flour for enhancement of vitamin and mineral intakes and nutrition status in northwest Chinese villages. Food Nutr Bull. 2012;33(2):161-8.
17. Varea A, Malpeli A, Disalvo L, Apezteguia M, Falivene M, Ferrari G, et al. Evaluation of the impact of a food program on the micronutrient nutritional status of Argentinean lactating mothers. Biol Trace Elem Res. 2012;150(1-3):103-8.
18. Hertrampf E, Cortes F, Erickson JD, Cayazzo M, Freire W, Bailey LB, et al. Consumption of folic acid-fortified bread improves folate status in women of reproductive age in Chile. J Nutr. 2003;133(10):3166-9.
19. Engle-Stone R, Nankap M, Ndjebayi AO, Allen LH, Shahab-Ferdows S, Hampel D, et al. Iron, Zinc, Folate, and Vitamin B-12 Status Increased among Women and Children in Yaounde and Douala, Cameroon, 1 Year after Introducing Fortified Wheat Flour. The Journal of nutrition. 2017;147(7):1426-36.
20. Tazhibayev S, Dolmatova O, Ganiyeva G, Khairov K, Ospanova F, Oyunchimeg D, et al. Evaluation of the potential effectiveness of wheat flour and salt fortification programs in five Central Asian countries and Mongolia, 2002-2007. Food Nutr Bull. 2008;29(4):255-65.
21. Liu S, West R, Randell E, Longerich L, O'Connor K S, Scott H, et al. A comprehensive evaluation of food fortification with folic acid for the primary prevention of neural tube defects. BMC Pregnancy Childbirth. 2004;4(1):20.
22. Colapinto CK, O'Connor DL, Tremblay MS. Folate status of the population in the Canadian Health Measures Survey. CMAJ. 2011;183(2):E100-6.
23. Brito A, Mujica-Coopman MF, López de Romaña D, Cori H, Allen LH. Folate and vitamin B12 status in Latin America and the Caribbean: an update. Food Nutr Bull. 2015;36 Suppl 2:S109-18.
24. Chen LT, Rivera MA. The Costa Rican experience: reduction of neural tube defects following food fortification programs. Nutr Rev. 2004;62(6 Pt 2):S40-3.
25. Schultz JT, Vatucawaqa PT. Impact of iron fortified flour in child bearing age (CBA) women in Fiji. 2010 report. Suva, Fiji: National Food and Nutrition Centre; 2012. Available from: http://www.nutrition.gov.fj/wp-content/uploads/2013/02/ImpactOfIronFortifiedFlourInCBAWomen-2010Report.pdf
26. Abdollahi Z, Elmadfa I, Djazayeri A, Sadeghian S, Freisling H, Mazandarani FS, et al. Folate, vitamin B12 and homocysteine status in women of childbearing age: baseline data of folic acid wheat flour fortification in Iran. Ann Nutr Metab. 2008;53(2):143-50.
27. Modjadji S, Alberts M, Mamabolo R. Folate and iron status of South African non-pregnant rural women of childbearing age, before and after fortification of foods. South Afr J Clin Nutr. 2007;20(3):89-93.
28. Pfeiffer CM, Hughes JP, Lacher DA, Bailey RL, Berry RJ, Zhang M, et al. Estimation of trends in serum and RBC folate in the U.S. population from pre- to postfortification using assay-adjusted data from the NHANES 1988-2010. J Nutr. 2012;142(5):886-93.
29. Crider KS, Bailey LB, Berry RJ. Folic acid food fortification-its history, effect, concerns, and future directions. Nutrients. 2011;3(3):370-84.
30. Odewole OA, Williamson RS, Zakai NA, Berry RJ, Judd SE, Qi YP, et al. Near-elimination of folate-deficiency anemia by mandatory folic acid fortification in older US adults: Reasons for Geographic and Racial Differences in Stroke study 2003-2007. Am J Clin Nutr. 2013;98(4):1042-7.
31. Guralnik JM, Eisenstaedt RS, Ferrucci L, Klein HG, Woodman RC. Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. Blood. 2004;104(8):2263-8.
32. Ganji V, Kafai MR. Hemoglobin and hematocrit values are higher and prevalence of anemia is lower in the post-folic acid fortification period than in the pre-folic acid fortification period in US adults. Am J Clin Nutr. 2009;89(1):363-71.
33. Fortmann SP, Burda BU, Senger CA, Lin JS, Beil TL, O'Connor E, et al. Vitamin, mineral, and multivitamin supplements for the primary prevention of cardiovascular disease and cancer: a systematic evidence review for the US Preventive Services Task Force. U.S. Preventive Services Task Force Evidence

Syntheses, formerly Systematic Evidence Reviews. Rockville (MD): Agency for Healthcare Research and Quality (US); 2013.
34. Huo Y, Li J, Qin X, Huang Y, Wang X, Gottesman RF, et al. Efficacy of folic acid therapy in primary prevention of stroke among adults with hypertension in China: the CSPPT randomized clinical trial. JAMA. 2015;313(13):1325-35.
35. Yang HT, Lee M, Hong KS, Ovbiagele B, Saver JL. Efficacy of folic acid supplementation in cardiovascular disease prevention: an updated meta-analysis of randomized controlled trials. Eur J Intern Med. 2012;23(8):745-54.
36. Morris MS, Jacques PF, Rosenberg IH, Selhub J. Folate and vitamin B-12 status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification. Am J Clin Nutr. 2007;85(1):193-200.
37. Miller JW, Garrod MG, Allen LH, Haan MN, Green R. Metabolic evidence of vitamin B-12 deficiency, including high homocysteine and methylmalonic acid and low holotranscobalamin, is more pronounced in older adults with elevated plasma folate. Am J Clin Nutr. 2009;90(6):1586-92.
38. Castillo-Lancellotti C, Margozzini P, Valdivia G, Padilla O, Uauy R, Rozowski J, et al. Serum folate, vitamin B12 and cognitive impairment in Chilean older adults. Public Health Nutr. 2015:1-9.
39. Moore EM, Ames D, Mander AG, Carne RP, Brodaty H, Woodward MC, et al. Among vitamin B12 deficient older people, high folate levels are associated with worse cognitive function: combined data from three cohorts. J Alzheimers Dis. 2014;39(3):661-8.
40. Clarke R, Sherliker P, Hin H, Molloy AM, Nexo E, Ueland PM, et al. Folate and vitamin B12 status in relation to cognitive impairment and anaemia in the setting of voluntary fortification in the UK. Br J Nutr. 2008;100(5):1054-9.
41. Mills JL, Carter TC, Scott JM, Troendle JF, Gibney ER, Shane B, et al. Do high blood folate concentrations exacerbate metabolic abnormalities in people with low vitamin B-12 status? Am J Clin Nutr. 2011;94(2):495-500.
42. MacFarlane AJ, Greene-Finestone LS, Shi Y. Vitamin B-12 and homocysteine status in a folate-replete population: results from the Canadian Health Measures Survey. Am J Clin Nutr. 2011;94(4):1079-87.
43. Carmel R. Does high folic acid intake affect unrecognized cobalamin deficiency, and how will we know it if we see it? Am J Clin Nutr. 2009;90(6):1449-50.
44. Berry RJ, Carter HK, Yang Q. Cognitive impairment in older Americans in the age of folic acid fortification. Am J Clin Nutr. 2007;86(1):265-7; author reply 7-9.
45. Yang Q, Cogswell ME, Hamner HC, Carriquiry A, Bailey LB, Pfeiffer CM, et al. Folic acid source, usual intake, and folate and vitamin B-12 status in US adults: National Health and Nutrition Examination Survey (NHANES) 2003-2006. Am J Clin Nutr. 2010;91(1):64-72.
46. Ministry for Primary Industries. Scientific evaluation of comments on submissions received on the future of folic acid fortification in New Zealand. MPI technical paper No. 2012/25. Wellington: MPI; 2012. Available from: https://www.mpi.govt.nz/document-vault/4048.
47. Qi YP, Do AN, Hamner HC, Pfeiffer CM, Berry RJ. The prevalence of low serum vitamin B-12 status in the absence of anemia or macrocytosis did not increase among older U.S. adults after mandatory folic acid fortification. J Nutr. 2014;144(2):170-6. doi: 10.3945/jn.113.183095.
48. Mills JL, Von Kohorn I, Conley MR, Zeller JA, Cox C, Williamson RE, et al. Low vitamin B-12 concentrations in patients without anemia: the effect of folic acid fortification of grain. Am J Clin Nutr. 2003;77(6):1474-7.