Supplemental Material: Annu. Rev. Nutr. 2022. 42:423-452
https://doi.org/10.1146/annurev-nutr-043020-091647
Folic Acid and the Prevention of Birth Defects: 30 Years of Opportunity and Controversies
Crider, Ping, Yeung, Mai, Zauche, Wang, Daniels, and Williams

Supplemental Appendix 1. Food Vehicle and Folic Acid Fortification Dosage Levels in Countries with Mandatory Folic Acid Fortification Programs

Country	Food Source	Food Source Description	Dosage Level (mcg/100g)	Implementation Status (Year)
Afghanistan	Wheat flour	The roller milling of wheat flour to produce a refined white flour. Wheat flours are considered to be one of the most cost effective, technically feasible and widely used vehicles for B group vitamins, folic acid, Vitamin A and minerals through centralized milling. Wheat flour products, especially bread and biscuits are also consumed by most sectors of the population.	100 mcg/100g	2014
Argentina	Wheat flour	Enriched flour used for the preparation of food products as established by law and this regulation, with exception of dietetic products and flours destined for export, flours destined to elaborate products for export and those of productions contemplated in Law No.25,127 of Ecological, Biological or Organic Production	220 mcg/100g	2002
Australia	Wheat flour	Wheat flour for making bread must contain no less than 2 mg/kg and no more than 3 mg/kg of folic acid; and no less than 6.4 mg/kg of thiamin. Wheat flour for making bread; does not include: (a) pizza bases; (b) breadcrumbs; (c) pastries; (d) cakes, including but not limited to brioche, panettone and stollen; (e) biscuits; or (f) crackers.	250 mcg/100g	2015
Bahrain	Wheat flour	White wheat flour	150 mcg/100g	2002
Belize	Wheat flour	This standard applies to wheat flour (also called flour, white flour, enriched flour) and whole wheat flour that is intended for human consumption, made from clean, sound and marketable grades of millable wheat grains of the species Triticum aestivum L. (common wheat) or from Triticum compactum Host, (club wheat).	180 mcg/100g	1998

Country	Food Source	Food Source Description	Dosage Level (mcg/100g)	Implementation Status (Year)
		It does not apply to: a) Any product made from durum wheat (hard wheat, T. durum Desf.); b) Whole meal flour or semolina made from common wheat or club wheat, or mixtures thereof; c) Wheat flour to be used in brewing, for the production of starch or gluten, or for non-food use; d) Wheat flour in which the protein content has been reduced; e) Wheat flour subjected to special treatments other than bleaching or dying; or f) Wheat flour containing ingredients not mentioned in Section 4.2 (such as self-rising flour).		
Benin	Wheat flour	Cereals - wheat bread flour enriched with iron and folic acid	250 mcg/100g	2012
Bolivia	Wheat flour	--	150 mcg/100g	1997
Brazil	Wheat flour	--	180 mcg/100g	2002
	Maize flour	Cornmeal and corn flakes	180 mcg/100g	2002
Burkina Faso	Wheat flour	--	250 mcg/100g	2012
Burundi	Wheat flour	This decree applies to the compulsory fortification of staple foods following: 1- Corn flour; 2- Cassava flour; 3- Wheat flour; 4- Edible vegetable oil and fat; 5- Any other product than the Ministry having public health in its attributions will have declared by publication in the Official Bulletin of Burundi (BOB), product referred to for the purposes of this decree	230 mcg/100g	2015
	Maize flour	This decree applies to the compulsory fortification of staple foods following:	120 mcg/100g	2015

Country	Food Source	Food Source Description	Dosage Level (mcg/100g)	Implementation Status (Year)
		1- Corn flour; 2- Cassava flour; 3- Wheat flour; 4- Edible vegetable oil and fat; 5- Any other product than the Ministry having public health in its attributions will have declared by publication in the Official Bulletin of Burundi (BOB), product referred to for the purposes of this decree		
Cabo Verde	Wheat flour	Cereals	260 mcg/100g	2014
Cameroon	Wheat flour	Wheat flour delivered in bulk or prepackaged and ready for sale to consumers or intended for use in ordinary breadmaking, special breadmaking, pastries, pastry and biscuits	500 mcg/100g	2011
Canada	Wheat flour	White flour, enriched flour or enriched white flour	150 mcg/100g	1998
Chile	Wheat flour	Wheat flour tailored on bread consumption	180 mcg/100g	2000
Colombia	Wheat flour	Wheat flour that is marketed in the national territory for direct sale to the consumer, such as for the manufacture of bakery products, pastry, biscuits, pasta, and others	154 mcg/100g	1996
Costa Rica	Wheat flour	Wheat flour used in the country for human consumption, whether for home use or in the food industry	180 mcg/100g	1997
	Maize flour	Enriched corn meal for human consumption in the country, whether it is of national production, donated or imported	130 mcg/100g	1999
	Rice	Long-type milled rice, except for the gourmet type, which is used for direct human consumption in the country, whether it is nationally produced, donated and imported	180 mcg/100g	2001
Cote d'Ivoire	Wheat flour	Enriched wheat flour meant for bread-making wheat flour intended for human consumption under tariff heading	150 mcg/100g	2007

Country	Food Source	Food Source Description	Dosage Level (mcg/100g)	Implementation Status (Year)
Cuba	Wheat flour	--	185 mcg/100g	2012
Djibouti	Wheat flour	Fortified wheat flour is understood to mean any flour intended for human consumption	130 mcg/100g	2013
Dominican Republic	Wheat flour	Most of the wheat flour is used for bread and pasta, with a small percentage for crackers and all-purpose uses	180 mcg/100g	2009
Ecuador	Wheat flour	--	170 mcg/100g	1996
El Salvador	Wheat flour	Fortified wheat flour for human consumption, made with common wheat, Triticum aestivum L. or branched wheat, Triticum compactum Host , or a mixture thereof, in bulk or prepackaged and that is ready for use	180 mcg/100g	2007
	Maize flour	Nixtamalized corn flour: the dehydrated product obtained from grinding of corn kernels (Zea mays) subjected to partial cooking with water in the presence of hydroxidecalcium	100 mcg/100g	2009
Fiji	Wheat flour	Wheat flour for direct human consumption prepared from common wheat, Triticum aestivum L., or club wheat, Triticum compactum Host., or mixtures thereof, by grinding or milling processes in which the bran and germ are partly removed and the remainder is comminuted to a suitable degree of fineness, and which is prepackaged ready for sale to the consumer or destined for use in other food products	200 mcg/100g	2009
Gambia	Wheat flour	--	260 mcg/100g	2020
Ghana	Wheat flour	Cereals and pulses-specification for fortified soft and strong wheat flour	208 mcg/100g	2010

Country	Food Source	Food Source Description	Dosage Level (mcg/100g)	Implementation Status (Year)
Guatemala	Wheat flour	Fortified wheat flour for human consumption, made with common wheat, Triticum aestivum L. or branched wheat, Triticum compactum Host , or a mixture thereof, in bulk or prepackaged and that is ready for use	40 mcg/100g	2007
	Maize flour	Corn dough or corn flour for tortillas	135 mcg/100g	2015
Guinea	Wheat flour	--	135 mcg/100g	2006
Haiti	Wheat flour	Wheat flour is consumed in several forms including in soups, porridge, pasta, bakery products and pastries and snacks (bread, candies, cookies, pâtés and other sweet or savory fried dough)	--	2017
Honduras	Wheat flour	--	180 mcg/100g	2007
Indonesia	Wheat flour	--	200 mcg/100g	2002
Iran	Wheat flour	--	150 mcg/100g	2007
Jordan	Wheat flour	--	152 mcg/100g	2002
Kazakhstan	Wheat flour	--	140 mcg/100g	2006
Kenya	Wheat flour	Packaged wheat flour	150 mcg/100g	2012
	Maize flour	Packaged dry milled maize products	150 mcg/100g	2012
Kiribati	Wheat flour	Wheat flour for direct human consumption prepared from common wheat, Triticum aestivum L., or club wheat, Triticum compactum Host., or mixtures thereof, by grinding or milling processes in which the bran and germ are partly removed and the remainder is comminuted to a suitable degree of fineness, and which is pre-packaged ready for sale to the consumer or destined for use in other food products	200 mcg/100g	2011
Kosovo	Wheat flour	--	150 mcg/100g	2012

Country	Food Source	Food Source Description	Dosage Level (mcg/100g)	Implementation Status (Year)
Kyrgyzstan	Wheat flour	First grade wheat flour	100 mcg/100g	2015
Liberia	Wheat flour	--	260 mcg/100g	2017
Malawi	Wheat flour	Wheat flour for bread	200 mcg/100g	2017
	Maize flour	--	100 mcg/100g	2017
Mali	Wheat flour	--	250 mcg/100g	--
Mauritania	Wheat flour	--	--	2010
Mexico	Wheat flour	--	200 mcg/100g	1999
	Maize flour	Corn flour, nixtamalized corn flour, to the dehydrated product obtained from the grinding of the kernels of nixtamalized corn	200 mcg/100g	2008
Republic of Moldova	Wheat flour	--	140 mcg/100g	2012
Mongolia	Wheat flour	--	130 mcg/100g	2018
Morocco	Wheat flour	Flours resulting from the crushing of common wheat - with the exception of whole meal flour	100 mcg/100g	2005
Mozambique	Wheat flour	Bread	200 mcg/100g	2016
	Maize flour	--	200 mcg/100g	2016
Nepal	Wheat flour	--	150 mcg/100g	2011
New Zealand[1]	Wheat flour	Non-organic wheat flour used for bread making	200–300 mcg/100g	2023
Nicaragua	Wheat flour	--	180 mcg/100g	2007
	Rice	Rice for national consumption	100 mcg/100g	2011
Niger	Wheat flour	--	250 mcg/100g	2012
Nigeria	Wheat flour	--	260 mcg/100g	2014
	Maize flour	--	260 mcg/100g	2010

Country	Food Source	Food Source Description	Dosage Level (mcg/100g)	Implementation Status (Year)
Oman	Wheat flour	--	150 mcg/100g	1996
Palestine	Wheat flour	Wheat supplier warrants that the wheat flour shall conform to Codex Stan 152-1985, be freshly milled, suitable for bread making	150 mcg/100g	2010
Panama	Wheat flour	Wheat flour only, and not for products made of wheat flour such as cookies, crackers, etc.	180 mcg/100g	2003
Panama	Rice	White rice	100 mcg/100g	2009
Paraguay	Wheat flour	--	300 mcg/100g	1998
Peru	Wheat flour	--	120 mcg/100g	2006
	Rice	--	120 mcg/100g	2021
Rwanda	Wheat flour	--	215 mcg/100g	2020
	Maize flour	--	120 mcg/100g	2020
Senegal	Wheat flour	Soft wheat flour enriched with iron and folic acid	250 mcg/100g	2009
Solomon Islands	Wheat flour	Wheat flour for direct human consumption prepared from common wheat, Triticum eastivum L., or club wheat, Triticum compactum host., or mixtures thereof, by grinding or milling processes in which the bran and germ are partly removed and the remainder is comminuted to a suitable degree of fineness, and which is prepackaged ready for sale to the consumer or destined for use in other food products	200 mcg/100g	2010
	Rice	--	110 mcg/100g	2018
South Africa	Wheat flour	--	142.86 mcg/100g	2003
	Maize flour	Unshifted maize meal	200 mcg/100g	2003
United Republic of Tanzania	Wheat flour	--	300 mcg/100g	2011
	Maize flour	--	150 mcg/100g	2011

Country	Food Source	Food Source Description	Dosage Level (mcg/100g)	Implementation Status (Year)
Togo	Wheat flour	--	260 mcg/100g	--
Turkmenistan	Wheat flour	--	150 mcg/100g	2006
Uganda	Wheat flour	--	230 mcg/100g	2011
	Maize flour	Milled maize product, including maize meal and maize flour	100 mcg/100g	2011
United States of America[2]	Wheat flour	Enriched flour; enriched bread, rolls and buns, enriched flour; enriched self-rising flour, enriched farina	154 mcg/100g	1998
	Maize flour	Enriched corn meal and corn grits	187 mcg/100g	1998
	Rice	Enriched white and brown rice	231 mcg/100g	1998
Uruguay	Wheat flour	Enriched or fortified wheat flour	240 mcg/100g	2006
Uzbekistan	Wheat flour	First-grade wheat flour	120 mcg/100g	2011
Viet Nam	Wheat flour	--	511 mcg/100g	--
Yemen	Wheat flour	--	150 mcg/100g	2001
Zimbabwe	Wheat flour	--	200 mcg/100g	2016
	Maize flour	--	130 mcg/100g	2016

Data sources: https://fortificationdata.org, FAO (http://www.fao.org/faolex/country-profiles/en/ referenced 10/2021), WHO (https://extranet.who.int/nutrition/gina/en/policies/summary). For fortification regulations that allow a range for fortificant levels, this table presents the median fortificant level.

[1]Source: https://www.mpi.govt.nz/food-safety-home/nutrients-added-food/folate-and-the-addition-of-folic-acid-to-food

[2]The original US regulation for folic acid fortification was 140 mcg/100g, when FDA last updated Code of Federal Regulations the units for folic acid were denoted as 0.7 milligrams per pound, which converts to 154 mcg/100g.

Supplemental Appendix 2. Literature search for Figure 1

EMBASE was the only database that was searched to provide information for the NTD evidence hierarchy (**Figure 1**). The search was restricted by English language and the database was searched from inception to October 13, 2021. The search strategy was developed using the PUBMED database as the primary database. The search strategy contained various terms for folic acid, fortification, supplementation, and neural tube defects, and was filtered by a range of study design types (systematic reviews, meta-analyses, RCTs, cohort studies, case reports, animal studies, editorials etc.)

Supplemental Appendix 3. Literature search for Figure 3

For Figure 3A, studies were identified in a previously conducted systematic literature search (26). We included studies from countries with a food fortification program and included NTD rates or available numerator (number of reported NTD cases) and denominator data (number of births in the study) for both a pre-and post-fortification period. We excluded data where the post-fortification period was less than 2 years. Fortification levels were populated from Appendix 1.

For Figures 3B and 3C, the search strategy was developed to identify observationally and before and after studies evaluating the national and subnational fortification programs. The intervention of interest was fortification with folic acid alone. EMBASE and MEDLINE were the only databases that were searched, and searches were restricted by the English language. The databases were searched from inception to October 1, 2020. The search strategy was developed using the PUBMED database as the primary database and contained folic acid, fortified food, blood concentration, and bioassay terms. A particular challenge in constructing this search strategy was that there were no MeSH terms directly corresponding to ‘red blood cell folate’, ‘serum folate’, and ‘plasma folate’. Therefore, these terms were free-text terms in the search strategy and may have resulted in fewer studies being identified by the search.

Appendix 4: References for Results in the Figure 3

1. Amarin ZO, Obeidat AZ. 2010. Effect of folic acid fortification on the incidence of neural tube defects. Paediatr Perinat Epidemiol 24:349-51
2. Bar-Oz B, Koren G, Nguyen P, Kapur BM. 2008. Folate fortification and supplementation--are we there yet? Reprod Toxicol 25:408-12
3. Barboza-Argüello Mde L, Umaña-Solís LM, Azofeifa A, Valencia D, Flores AL, et al. 2015. Neural tube defects in Costa Rica, 1987-2012: origins and development of birth defect surveillance and folic acid fortification. Matern Child Health J 19:583-90
4. Chen LT, Rivera MA. 2004. The Costa Rican experience: reduction of neural tube defects following food fortification programs. Nutr Rev 62:S40-3
5. Cortes F, Mellado C, Pardo RA, Villarroel LA, Hertrampf E. 2012. Wheat flour fortification with folic acid: changes in neural tube defects rates in Chile. Am J Med Genet A 158A:1885-90
6. De Wals P, Rusen ID, Lee NS, Morin P, Niyonsenga T. 2003. Trend in prevalence of neural tube defects in Quebec. Birth Defects Res A Clin Mol Teratol 67:919-23
7. Dietrich M, Brown CJ, Block G. 2005. The effect of folate fortification of cereal-grain products on blood folate status, dietary folate intake, and dietary folate sources among adult non-supplement users in the United States. J Am Coll Nutr 24:266-74
8. Ebrahimi S, Ashkani-Esfahani S, Bagheri F. 2013. Prevalence of neural tube defects in Yasuj, Southwest Iran. Shiraz E-Medical Journal 14:1-9
9. Engle-Stone R, Nankap M, Ndjebayi AO, Allen LH, Shahab-Ferdows S, et al. 2017. 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. J Nutr 147:1426-36

10. Hertrampf E, Cortes F, Erickson JD, Cayazzo M, Freire W, et al. 2003. Consumption of folic acid-fortified bread improves folate status in women of reproductive age in Chile. J Nutr 133:3166-9

11. Knoops KT, Spiro A, 3rd, de Groot LC, Kromhout D, van Staveren WA, Tucker KL. 2009. Do dietary patterns in older men influence change in homocysteine through folate fortification? The Normative Aging Study. Public Health Nutr 12:1760-6

12. Liu S, West R, Randell E, Longerich L, O'Connor K S, et al. 2004. A comprehensive evaluation of food fortification with folic acid for the primary prevention of neural tube defects. BMC Pregnancy Childbirth 4:20

13. Lopez-Camelo JS, Castilla EE, Orioli IM, Inagemp, Eclamc. 2010. Folic acid flour fortification: impact on the frequencies of 52 congenital anomaly types in three South American countries. Am J Med Genet A 152A:2444-58

14. Lopez-Camelo JS, Orioli IM, da Graca Dutra M, Nazer-Herrera J, Rivera N, et al. 2005. Reduction of birth prevalence rates of neural tube defects after folic acid fortification in Chile. Am J Med Genet A 135:120-5

15. Malpeli A, Ferrari MG, Varea A, Falivene M, Etchegoyen G, et al. 2013. Short-term evaluation of the impact of a fortified food aid program on the micronutrient nutritional status of Argentinian pregnant women. Biol Trace Elem Res 155:176-83

16. Nazer HJ, Cifuentes OL. 2013. [Effects of wheat flour fortification with folic acid on the prevalence of neural tube defects in Chile]. Rev Med Chil 141:751-7

17. Persad VL, Van den Hof MC, Dube JM, Zimmer P. 2002. Incidence of open neural tube defects in Nova Scotia after folic acid fortification. CMAJ 167:241-5

18. Pfeiffer CM, Hughes JP, Lacher DA, Bailey RL, Berry RJ, et al. 2012. 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 142:886-93

19. Ray JG, Meier C, Vermeulen MJ, Boss S, Wyatt PR, Cole DE. 2002. Association of neural tube defects and folic acid food fortification in Canada. Lancet 360:2047-8

20. Ray JG, Vermeulen MJ, Boss SC, Cole DE. 2002. Declining rate of folate insufficiency among adults following increased folic acid food fortification in Canada. Can J Public Health 93:249-53

21. Ricks DJ, Rees CA, Osborn KA, Crookston BT, Leaver K, et al. 2012. Peru's national folic acid fortification program and its effect on neural tube defects in Lima. Rev Panam Salud Publica 32:391-8

22. Sanabria Rojas H, Tarqui-Mamani C, Arias Pachas J, Lam Figueroa N. 2013. Impact of fortifying wheat flour with folic acid on neural tube defects in Lima, Peru. Anales de la Facultad de Medicina 75:175-80

23. Santos LM, Lecca RC, Cortez-Escalante JJ, Sanchez MN, Rodrigues HG. 2016. Prevention of neural tube defects by the fortification of flour with folic acid: a population-based retrospective study in Brazil. Bull World Health Organ 94:22-9

24. Sayed AR, Bourne D, Pattinson R, Nixon J, Henderson B. 2008. Decline in the prevalence of neural tube defects following folic acid fortification and its cost-benefit in South Africa. Birth Defects Res A Clin Mol Teratol 82:211-6

25. Williams J, Mai CT, Mulinare J, Isenburg J, Flood TJ, et al. 2015. Updated estimates of neural tube defects prevented by mandatory folic Acid fortification - United States, 1995-2011. MMWR. Morbidity and mortality weekly report 64:1-5

26. Zaganjor I, Sekkarie A, Tsang BL, Williams J, Razzaghi H, et al. 2016. Describing the Prevalence of Neural Tube Defects Worldwide: A Systematic Literature Review. PLoS One 11:e0151586