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REVIEW

Global strategies for the prevention of neural tube defects through the improvement of folate status in women of reproductive age

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Abstract

Introduction Neural tube defects represent a global public health problem, mainly in countries where effective prevention strategies are not yet in place. The global prevalence of neural tube defects is estimated at 18.6/10,000 (uncertainty interval: 15.3–23.0) live births, where ~ 75% of cases result in under-five mortality. Most of the mortality burden is in low- and middle-income countries. The main risk factor for this condition is insufficient folate levels in women of reproductive age.

Methods This paper reviews the extent of the problem, including the most recent global information on folate status in women of reproductive age and the most recent estimates of the prevalence of neural tube defects. Additionally, we provide an overview of the available interventions worldwide to reduce the risk of neural tube defects by improving folate status in the population, including dietary diversification, supplementation, education, and fortification.

Results Large-scale food fortification with folic acid is the most successful and effective intervention to reduce the prevalence of neural tube defects and associated infant mortality. This strategy requires the coordination of several sectors, including governments, the food industry, health services providers, the education sector, and entities that monitor the quality of the service processes. It also requires technical knowledge and political will. An international collaboration between governmental and non-governmental organizations is essential to succeed in saving thousands of children from a disabling but preventable condition.

Discussion We propose a logical model for building a national-level strategic plan for mandatory LSFF with folic acid and explain the actions needed for promoting sustainable system-level change.

Keywords Neural tube defects · Prevention · Folic acid · Food fortification · Low- and middle-income countries

Abbreviations

CDC	Centers for disease control and prevention
EAR	Estimated average requirement
ETOPFA	Elective termination of pregnancy for fetal anomaly
FDA	Food and Drug Administration
HIC	High-income countries
LIC	Low-income countries
LMIC	Low- and middle-income countries
LSFF	Large-scale food fortification
MBA	Microbiological assay
MIC	Middle-income countries
NTD	Neural tube defects
RBC	Red blood cell
WHO	World Health Organization

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WIFAS Weekly iron and folic acid supplementation
WRA Women of reproductive age

Introduction

Neural tube defects (NTD) represent a failure of the neural tube to close properly in the early phase of gestation (around day 28 post-conception) [1]. The most common forms of NTD include spina bifida, encephalocele, and anencephaly. The latter is the most severe form, as this defect is incompatible with extrauterine life. Children presenting with other forms survive but often require neurosurgery to close the defect, either prenatally or postnatally. About 75% of them die before reaching the age of 5 years, mainly in low- and middle-income countries (LMIC) [2]. A common complication of spina bifida is hydrocephalus. Managing these patients requires early and recurrent neurosurgical, urological, and orthopedic follow-up, with multiple surgeries, hospitalizations, and clinic follow-ups [1]. These children live with a life-long disability that impacts them and their families. Given the scarcity of resources, this public health problem is especially burdensome in LMIC.

NTD have several causes, as summarized in Table 1. However, there is ample scientific evidence that the major contributing factor is insufficient folate levels, as reflected by red blood cell (RBC) folate. There is a dose–response relationship showing an inverse response between RBC folate and NTD risk [3, 4], with an inflection point of folate in RBC ~ 1000 nmol/L (determined by a microbiological assay or MBA, Table 2), above which risk of folate-responsive NTD-affected pregnancies is the lowest [3]. The World Health Organization (WHO) has identified a cut-off of 906 nmol/L of RBC folate at a population level to provide maximum protection against NTD [5]. The scientific evidence expresses population protection level as an NTD prevalence of 5–6 cases per 10,000 live births, as is found in every country that has implemented a successful large-scale food fortification (LSFF) program with folic acid [2, 6].

The objectives of this paper are (1) to review the extent of the problem, including the most recent global information on folate status in women of reproductive age and NTD prevalence estimates, (2) to provide an overview of available interventions to reduce NTD risk by improving folate status in the population, and (3) to propose activities that a country should consider in their strategic plan to establish a mandatory large-scale food fortification (LSFF) program with folic acid.

Identifying the extent of the problem

Assessment of folate status

RBC and serum/plasma folate levels are the most widely used biomarkers for assessing folate status. RBC folate is a biomarker of long-term folic acid intake, whereas serum/plasma folate reflects short-term folic acid intake [4, 15, 16]. RBC folate level is preferred to assess population-level folate status. The WHO recommends using a harmonized MBA to measure RBC folate to obtain reliable, comparable results across countries and time [5].

Folate *deficiency* increases the risk of developing megaloblastic anemia, with cut-off values of < 7 nmol/L for serum folate and < 227 nmol/L for RBC folate (Table 2) [5]. The folate concentration needed to support rapid cell division at the neural tube closure is much higher; therefore, it requires a higher cut-off to provide maximum protection against folate-dependent NTD. Folate levels below this cut-off are referred to as folate *insufficiency*. According to WHO guidelines, the most significant protective level against NTD is when RBC folate in the population reaches a threshold of 906 nmol/L or the corresponding value (748 nmol/L) when using a different calibrator for the MBA (Table 2) [16].

Table 1 Potential factors contributing to the occurrence of neural tube defects

Environmental exposures [7–10]	Infectious diseases [11]	Drugs [12]	Metabolic disorders [13]	Genetic susceptibility [14]	Nutritional factors [3]
Organic solvents Arsenic Pesticides Paints X-radiation	Malaria	Thalidomide Methotrexate Antiepileptics (carbamazepine, valproic acid) Antimalarials (sulfadiazine / pyrimethamine)	Diabetes mellitus Phenyl-ketonuria	Polymorphisms of the methylenetetrahydrofolate reductase (MTHFR) enzyme	Deficiency of B vitamins: riboflavin (B_2), pyridoxine (B_6), and B_{12} **Insufficiency of folate (B_9)**

Table 2 Deficiency and insufficiency of serum and RBC folate – definition and threshold values

Folate status	Associated outcome	Serum folate (nmol/L)	RBC folate[a] (nmol/L)
Deficiency	Megaloblastic anemia	<7[b] [5]	<227[b] [5]
	Elevated risk of megaloblastic anemia	<7[b] [18]	<305[b] [18]
Insufficiency	Elevated risk of neural tube defects	<25.5 [19]	<906[c] [4] <748[d] [20]

RBC red blood cell

[a]As recommended by World Health Organization [5]/Centers for Disease Control and Prevention [21]

[b]Determined by a homogenized microbiological assay using wild-type strain and folic acid standard as calibrator

[c]Determined by a homogenized microbiological assay using a chloramphenicol-resistant strain of *Lactobacillus rhamnosus* and folic acid standard as calibrator

[d]Determined by a homogenized microbiological assay using a chloramphenicol-resistant strain of *Lactobacillus rhamnosus* and 5-methyl-tetrahydrofolate standard as calibrator

Folate concentration shows a dose–response relationship concerning NTD risk [17].

Current folate status in women of reproductive age

There is a dearth of information about folate status globally. A recent systematic review article by Rogers et al. (2018) identified all nationally representative surveys that reported folate deficiency or insufficiency worldwide between 2000 and 2014 [22]. Most (> 70%) of the available information represented high- or upper-middle-income countries. Overall, folate insufficiency prevalence was > 40% in most countries. This review highlighted several shortfalls present in understanding folate status globally. They included the paucity of data (only 39 countries had conducted this evaluation during the study period, providing 45 surveys overall), scarcity of data in LMIC, scarcity of information on folate insufficiency, and heterogeneity of laboratory methods used (only ten surveys used the recommended harmonized MBA to measure RBC folate). The main caveat was related to the laboratory methods used to measure folate status, which required the authors to devise an "assay factor" to assess whether the survey was likely to overestimate, underestimate, or was likely to report correct results, taking the MBA (calibrated with 5-methyl-THF) recommended by the Centres for Disease Control and Prevention (CDC) as reference (Table 3).

Prevalence of NTD

Estimating NTD prevalence in LMIC has proved difficult and, in most cases, imprecise, often resulting in an underestimation of true values. This challenge is due to multiple factors; most LMICs do not have high-quality birth defects surveillance programs, including information on stillbirths and abortions. Additionally, elective termination of pregnancy for fetal anomalies (ETOPFA) is illegal in many LMICs, so the prevalence is measured by the rate of NTD cases out of 10,000 live births. Most recent estimates of NTD prevalence come from a systematic analysis by Blencowe et al. (2018) that estimated global prevalence based on available country birth registries and published literature [2]. This analysis showed that in 2015, there were an estimated 260,100 (uncertainty interval (UI): 213,800–322,000) new NTD globally (prevalence 18.6/10,000 (UI:15.3–23.0) live births). Out of this, 75% of cases (117,900) resulted in under-five mortality, with a majority in LMIC. The authors also report NTD prevalence rates per 10,000 live births without folic acid fortification by region: Southern Asia (31.96), East Asia (19.44), Northern Africa and Western Asia (17.45), and Sub-Saharan Africa (15.27) [2].

NTD prevention strategies

The following sections summarize the strategies available to improve folate status, highlighting their strengths and caveats. Figure 1 illustrates the interconnectivity and complementarity of the preventive strategies.

Dietary diversification

Naturally occurring folate is found in different foods, including green leafy vegetables (folic in Latin means leaf), beans, peas, lentils, asparagus, broccoli, beets, avocado, nuts and seeds, wheat germ, in some fruits (papaya, bananas, citrus fruits), and animal products like eggs and beef liver. While nutrition education at the individual or the community levels should include information on sources of folate and reasons to include these foods in the diet, it may be challenging to obtain the required daily intake of folate from only food. Current folic acid daily dose recommendations build on folate intake provided by a regular diet [23].

Table 3 Global folate deficiency and insufficiency in women of reproductive age (2000–2014)[a]

Country classification by income level	Range of folate deficiency prevalence in women of reproductive age[b]	Range of folate insufficiency prevalence[c] in women of reproductive age
High-income	0–11%	23–73%
Upper-middle income	2–19%	No reliable data[d]
Lower-middle income	7–49%	47–98%
Low-income	18–79%	Not measured

Data source: Rogers et al. [22]

[a]Reporting "likely correct prevalence" as defined by the authors based on pre-specified values of the ratio of survey assay to the microbiological assay proposed by the US Centers for Disease Control and Prevention

[b]Prevalence based on red blood cell folate < 305 nmol/L using folic acid calibrator or serum folate < 7 nmol/L using folic acid calibrator, considering only those surveys reporting on a likely correct assessment of folate deficiency prevalence

[c]Prevalence based on red blood cell folate < 906 nmol/L using folic acid calibrator, considering only those surveys reporting on a likely correct assessment of folate insufficiency prevalence

[d]Prevalence of folate insufficiency in women of reproductive age in upper-middle-income countries was based on only two surveys; one likely overestimated prevalence and one likely underestimated prevalence

Periconceptional oral folic acid supplementation for primary prevention

In the early 1980s, Smithells et al. provided the earliest evidence of the efficacy of primary prevention of NTD with multivitamin supplementation, including folic acid [24–26]. Later studies identified folic acid as the primary contributor to the preventive effect [27–30]. A systematic review of randomized or quasi-randomized clinical trials evaluating the effect of periconceptional folate supplementation in women, independent of age and parity, reporting on 7391 women (2033 with a history of an NTD-affected pregnancy and 5358 without NTD history) demonstrated that oral supplementation prevents the occurrence and recurrence of NTD by up to 70% [31].

WHO and the US Centers for Disease Control and Prevention (CDC) recommend that all women of reproductive age get 400 µg of folic acid daily and folate in the diet. For women with diabetes or receiving an anticonvulsant treatment, the daily recommended dose is 5000 µg, in addition to dietary advice [23].

A recent systematic review on the impact of folic acid fortification and supplementation policies worldwide determined that government supplementation campaigns or recommendations effectively reduced NTD prevalence [32]. They also highlighted the role of education and adherence to recommendations. However, the unintended pregnancy rate in LMIC is high and is negatively associated with income compared to HIC. In 2015–2019, the unintended pregnancy rate for HIC was 34 per 1000 women of 15–49 years, for middle-income countries (MIC) was 66 per 1000 and for LIC was 96 per 1000 [33]. There is a potential disparity in those who access prenatal care. Women seeking such care may be more affluent, more educated, or have a history of complicated pregnancies [34, 35]. Supplementation is not an effective strategy for unintended pregnancies [33].

Periconceptional oral folic acid supplementation for recurrence prevention

NTD recurrence is an NTD-affected pregnancy in a mother with a previously NTD-affected pregnancy. The American

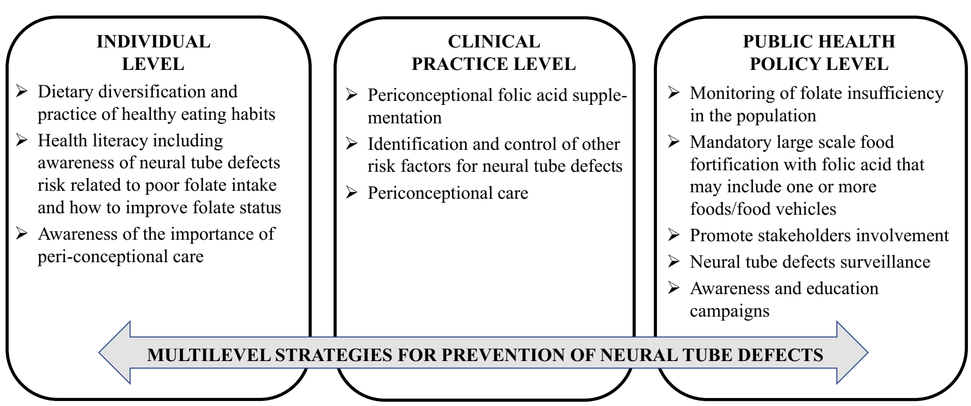


Fig. 1 Interconnected and complementary strategies for the prevention of neural tube defects. Source: authors' work using Microsoft's PowerPoint SmartArt

College of Medical Genetics, CDC, and the American College of Obstetricians and Gynecologists recommend intake of 4000 µg per day of folic acid, starting 1 month before the intended pregnancy and continuing for the first trimester of pregnancy to prevent NTD recurrence in the subsequent pregnancy [23, 36–38].

Grosse and Collins' (2007) systematic review reports a 4% recurrence risk after one previously NTD-affected pregnancy and 11.1% after two NTD-affected pregnancies from pooled data in Great Britain, North America, and Europe [39]. Four randomized trials focused on NTD recurrence prevention via folic acid supplements showed a pooled NTD rate of 0.6% compared to those who did not take folic acid supplements of 4.1%, a risk reduction of 87% [39]. Five observational studies provide a range of 85–100% recurrence risk reduction [39]. https://www.msn.com/en-ca/feed.

Folic acid weekly supplementation

No randomized controlled trials have tested the efficacy of weekly periconceptional folate supplementation, specifically on NTD prevention rates. However, three studies have documented improvements in folate status (as a surrogate for NTD prevention) in response to weekly folic acid supplements, with one documenting reductions in NTD following a pre-post analysis.

The first study enrolled 74 women from rural and urban areas in the state of Nuevo Leon, Mexico, including 39 who had a previously NTD-affected pregnancy and 35 who had a normal pregnancy. All women received 5000 µg (5 mg) of folic acid weekly for 3 months. Results showed that 90% of women significantly increased RBC and plasma folate concentrations [40]. Following these encouraging results, the state scaled up the intervention to include awareness education to the public, focusing on women affected by an NTD pregnancy (recurrence prevention). The program provided free supplementation to 250,000 low-income women of childbearing age. Follow-up at 28 months drew data from an active hospital-based NTD surveillance program, complemented by fetal and child mortality from death records. Of 209 women enrolled in the recurrence prevention intervention, 30 became pregnant, with 0 recurrences. The state-wide evaluation showed a significant decrease in NTD prevalence from 10.04/10,000 live births at baseline to 5.8/10,000 live births at 28 months [41].

A three-arm randomized control trial in New Zealand enrolled 114 women of reproductive age (WRA) for 12 weeks in a (2800 µg weekly, 400 µg daily, placebo daily) and assessed the effect of a once-a-week supplementation dose on RBC folate concentrations. Results showed a protective increase in folate levels in 49% of women, yet this was less effective compared to 74% of women in the daily 400 µg group [42].

More recently, a three-arm randomized controlled trial in Malaysia documented the response of 331 young women (median age 18 years), randomly assigned to receive weekly iron and folic acid supplementation or WIFAS in a dose of 60 mg iron and either 2800 µg 400 µg, or 0 µg, for 16 weeks. This trial found that two groups with folic acid showed a higher mean RBC than those receiving none, and those receiving the higher dose of folic acid (2800 µg) had a higher mean RBC folate than those receiving the lower (400 µg) dose (mean difference of 271 nmol/L). More importantly, women who received the 2800 µg were seven times ($RR = 7.3$, 95% CI:3.9–13.7) more likely to show RBC folate levels above the cut-off sufficiency point (748 nmol/L) [43].

Education campaigns on folic acid supplementation

Lack of knowledge and awareness of the protective effect of folic acid against NTD is the leading cause of inadequate folic acid intake among WRA [44]. Prenatal consultation is a valuable strategy for those who plan to conceive, but often it is not timely. Most women find out they are pregnant after 5 weeks when they miss their menstrual period. As neural tube closure occurs within the first 4 weeks post-conception, initiating the consumption of folic acid at this stage is too late. A total of 44% of pregnancies worldwide were unintended from 2010 to 2014 [45], and in LMIC, planned pregnancies are even lower among adolescents and women with limited access to information.

Furthermore, supplement use is deficient even among women planning their pregnancy. Werler et al. found that among 60% of planned pregnancies in the USA and Canada, only 8% of mothers with previously NTD-affected pregnancies and 13% in healthy pregnancies used folic acid supplements before getting pregnant [30]. Similarly, HIC demonstrate the same gap between supplement use in planned pregnancy: 3%/77% (UK) [46],14%/76% (Denmark) [47], and 31%/80% (Norway) [48].

A systematic review by Stockley et al. (2008) identified several challenges related to this strategy, including young age, ethnicity, and low socioeconomic status. Isolated educational campaigns that use printed resources or mass media were not effective long term, while healthcare-based initiatives could be effective as long as supplements were easily accessible. Most effective campaigns focus on women in a state of vulnerability, yet they reach only 50% of women [49].

Large-scale food fortification (LSFF)

LSFF refers to the process of adding essential micronutrients (vitamins and minerals) at the production stage (i.e., milling) to foods or other food vehicles (i.e., salt) widely consumed by the general population [50]. LSFF's primary goal is to improve the population's health status through increased nutrient intake without significant changes to habitual food consumption. LSFF is a population-level intervention and has several advantages: (1) no need to change diet at the individual level; (2) feasibility of fortifying foods with multiple micronutrients at once; (3) high return-on-investment on population-level health; and (4) safe [50].

LSFF can be especially effective in improving the population's nutritional status if well planned, executed, and maintained by respective governmental entities. LSSF does not replace other methods of food fortification such as targeted food fortification, point-of-use or home fortification, or biofortification but can work in combination with those. Effective implementation of LSFF programs implies efficacy and effectiveness research that provides evidence about the actual impact of LSSF on health outcomes [51].

LSFF with folic acid is successful and highly effective in increasing folate levels in WRA and reducing NTD prevalence in LMIC [52]. Table 4 summarizes the best available information from peer-reviewed publications on the effectiveness of LSFF with folic acid on NTD prevalence, NTD-associated mortality, and folate levels in LMIC and selected HIC countries.

As shown in Table 4, LSFF with folic acid is consistently successful and highly effective in increasing folate levels in WRA, leading to a reduced NTD prevalence, including NTD-associated infant mortality. Several MIC such as Costa Rica [53–55], Brazil [56, 57], Mexico [57], Argentina [58], Iran [59], South Africa [60], and Cameroon [61] have demonstrated such benefits after the implementation of mandatory fortification. Furthermore, NTD prevalence reduction ranged from 30 to 31% (Brazil and South Africa) [56, 58, 60] and 58–59% (Costa Rica, Mexico) [54, 57], mainly due to the fortification of wheat flour with folic acid at levels greater than 1.8 mg/kg or corn flour at 1.3–2 mg/kg. Costa Rica demonstrated one of the highest rates of reduction in NTD prevalence by fortifying different foods as a strategy to reach different populations, including corn and wheat flour (1.8 mg/kg), rice (1.8 mg/kg), and milk (400 µg/200 ml) [53, 54].

In China, NTD prevalence has remained high despite mandatory supplementation programs. However, a study in Shanxi province showed a 68% reduction in NTD prevalence in villages with mandatory wheat flour fortification compared to those without fortification [62].

Australia provides a singular case study, as there are published evaluations showing changes in NTD prevalence over time, corresponding to a pre-fortification period with no intervention (1980–1992), followed by the introduction of widely available folic acid supplementation (1993–1995), voluntary fortification (1996–2008), and lastly by mandatory fortification (September 2009). Comparing the no-intervention period with the promotion of the supplementation period, the prevalence of anencephaly, spine bifida, and encephalocele per 10,000 births remained significantly unchanged (PR of 1.1, 0.9, and 1.2, respectively). After voluntary fortification, anencephaly and spina bifida prevalence significantly reduced by 32% and 23%, respectively [71]. However, the reduction in NTD was only significant for the population of non-Aboriginal origin (Table 4). They achieved a significant decrease in NTD prevalence across all population groups (i.e., Aboriginal and Torres Strait Islanders) only after mandatory fortification. Hilder et al. [71] documented a drop from 10.2 per 10,000 births in the voluntary fortification period to 9.4 per 10,000 births in the mandatory fortification period ($RR = 0.86$, 95%CI 0.74–0.99). There was a higher reduction in the population of Aboriginal origin, from 19.6 per 10,000 to 5.1 per 10,000 births ($RR = 0.26$, 95%CI 0.12–0.55) [72]. These results agree with those reported by Brown et al. who demonstrated an 85% reduction in folate insufficiency (RBC less than 310 nmol/L) in a survey of public hospitals in Australia, which decreased from 3.4% after voluntary fortification to 0.5% after mandatory fortification [72].

Though data are scarce, the reduction in NTD-related mortality after mandatory LSFF ranged between 66% (South Africa) [60], 68% (Argentina) [56], and 71% (Costa Rica) [54].

Building a national-level strategic plan for mandatory LSFF with folic acid

Enacting, promulgating, and enforcing a national regulation for LSFF with folic acid is an attainable goal, but challenges may exist. Establishing a strategic plan requires the coordination of different sectors, including governments, the food industry, health services providers, the education sector, and entities that monitor the quality of the service processes. It also requires technical knowledge, political will, and partnership with national entities and supranational organizations. We describe the main elements of a strategic plan for a national-level mandatory LSFF, summarized in a logical model presented in Fig. 2.

Building the evidence to support LSFF

Establishing a mandatory food fortification program in a specific country or population must be based on three factors: (1) food/micronutrient intake, (2) micronutrient status (using the relevant biomarkers), and (3) identification of a suitable food vehicle for fortification [50, 73].

Dietary surveys tailored for each country or culture assess food consumption habits and folate intake. A fortification rapid assessment tool (FRAT) provides the necessary information about food consumption patterns at the household level. This tool combines a simplified 24-h recall and food consumption questionnaires [74, 75] to support an informed

Table 4 Impact of large-scale folic acid food fortification on neural tube defects (NTD) prevalence and folate status in women in low- and middle-income countries and high-income countries

Country (study coverage)	Fortified foods (folic acid amount)	Mode of fortification (year)	Observed outcomes [ref]	Pre-fortification	Post-fortification	Relative Risk (95%CI); observed change	Type of study, data source and observations
Low- and middle-income countries							
Costa Rica (National)	Wheat flour (1.5 mg/kg) (1.8 mg/kg) Maize flour (1.3 mg/kg) Milk (40 µg/250mL) Rice (1.8 mg/kg)	Mandatory (1997) Mandatory (2003) Mandatory (1999) Mandatory (2001) Mandatory (2002)	NTD prevalence [53]	Period: 1987–1991 9.8 per 10,000	Period: 2003–2012 4.8 per 10,000	0.49; ↓51% ***	Uncontrolled, before-and-after series. Prevalence data: National births defects register center (live births and stillbirths).(1.8 mg/kg).
			NTD prevalence [54]	Period: 1987–1997 12.0 per 10,000	Period: 2004–2009 5.4 per 10,000	0.42; ↓58% ***	
			NTD mortality rate [54]	Period: 1987–1997 6.4 per 10,000	Period: 2004–2009 1.9 per 10,000	0.29; ↓71% ***	Mortality data: National Institute of statistics and census (all population death certificates).
			Folate deficiency prevalence in WRA, [55]	Period: 1996 24.7	Period: 2008–2009 3.8	↓84% **	National Nutrition Surveys, population-based.
Brazil (regional data)	Wheat and Maize flour (1.5 mg/kg)	Mandatory (2004)	NTD prevalence [56]:	Period: 2001–2004	Period: 2005–2014		Uncontrolled, before-and-after series. Population-based data of stillbirths and live births from national information system in central, south-eastern, and southern Brazil. **Note:** reference period for RR is the post-fortification period.
			Overall	7.9 per 10,000	5.5 per 10,000	1.43 (1.38–1.50); ↓30.1%	
			Stillbirths	177.4 per 10,000	117 per 10,000	1.52 (1.40–1.63); ↓34% ***	
			Livebirths	5.7 per 10,000	4.4 per 10,000	1.29 (1.24–1.35); ↓22.8%***	
			NTD prevalence [57]	Period: 2003–2005 31.4 per 10,000	Period: 2005–2007 24.3 per 10,000	0.77 (0.64–0.91); ↓33%**	Systematic review. Data from different sources, mostly hospital-based registries.
Argentina	Wheat flour (2.20 mg/kg)	Mandatory (2002–2003)	NTD prevalence [58]:	Period: 2000	Period: 2005		Uncontrolled, before-and-after series. Data: hospital discharge statistics. Fetal and infant mortality rates data from Vital Statistics Series (birth and death certificates).
			SB	24.3 per 10,000	13.2 per 10,000	0.54(0.46–0.64); ↓45.6%***	
			Anencephaly	4.1 per 10,000	1.9 per 10,000	0.46(0.30–0.70); ↓53.7%***	
			Encephalocele	3.8 per 10,000	2.5 per 10,000	0.67(0.45–0.98); ↓33.4%	
			NTD mortality [58]:	Period: 2000–2004	Period: 2005–2006		
			SB (fetal and infant)	0.8 per 10,000	0.3 per 10,000	↓67.8%***	
			Anencephaly (fetal)	5.3 per 10,000	2.3 per 10,000	↓56%***	
Mexico RYVEMCE	Wheat flour (1.8 mg/kg)	Mandatory 2000	NTD prevalence [57]	Period: 1995–1999 35.8 per 10,000	Period: 2000–2006 14.7 per 10,000	0.41 (0.36–0.47); ↓59%***	Systematic review. Data from Mexico: 25 hospitals from Mexican External Malformations Epidemiological Surveillance Registry (RYVEMCE).

Table 4 (continued)

Country (study coverage)	Fortified foods (folic acid amount)	Mode of fortification (year)	Observed outcomes [ref]	Pre-fortification	Post-fortification	Relative Risk (95%CI); observed change	Type of study, data source and observations
Iran (Golestan)	Wheat flour (1.5 mg/kg)	Mandatory (Iran 2001 Golestan 2006)	Folate deficiency prevalence in WRA [59]	Period: 2006–2007 14.3	Period: 2007–2008 2.3	↓83% ***	Uncontrolled, before-and-after series. Folate data source: cross-sectional hospital-based surveys of 13361 postpartum women in Golestan. NTD prevalence data source: Hospital-based surveillance system in Golestan, including live births and stillbirths.
			Total folate intake in WRA [59]	Period: 2006–2007	Period: 2007–2008		
			NTD prevalence [59]	198.3 μg/day	413.7 μg/day	↑226 μg/day ***	
				Period: 2006–2007 31.6 per 10,000	Period: 2007–2008 21.9 per 10,000	0.09 (0.04–0.21); ↓31% ***	
South Africa (Four provinces)	Wheat flour (1.5 mg/kg) Maize flour (2.21 mg/kg)	Mandatory (2003)	NTD prevalence [60]:	Period: 2003–2004	Period: 2004–2005		Ecological study among 12 public hospitals in four provinces in South Africa. NTD prevalence data source: sentinel hospital-based surveillance system. Mortality data source: mortality system.
			Total	14.1 per 10,000	9.8 per 10,000	0.69 (0.49–0.98); ↓30,5%***	
			Anencephaly	4.1 per 10,000	3.7 per 10,000	0.89 (0.5–1.6); ↓10.9% ***	
			SB	9.3 per 10,000	5.4 per 10,000	0.58 (0.37–0.92); ↓41.6%	
			NTD Perinatal mortality [60]	Period: 2001–2003 4.19 per 10,000	Period: 2005–2006 1.43 per 10,000	0.34(0.25–0.47); ↓65.9%***	
China (Shanxi province)	Wheat flour for a community trial (2mg/kg)	Not mandatory	NTD prevalence [62]	Control group: 229.1 per 10,000	Intervention group: 72.9 per 10,000	0.31 (0.21–0.47); ↓68.2%***	Case-control study (regular flour *vs.* fortified flour), after a community intervention in eleven communities of Shanxi, a high rate of NTD province.
			Serum folate in WRA [62] mean ± SD	18.73 ±5.47	25.44± 7.72	***	
Cameroon (two urban cities)	Wheat flour (5mg/kg)	Mandatory (2011)	Plasma folate levels in WRA,prevalence [61]:	Period: 2009	Period: 2012		Regional Survey in 300 households in Yaoundé and Doalá. Include women of childbearing age and children, two years before and one year after fortification.
			<7 nmol/L	6.1±1.5	0.3±0.3	***	
			<10 nmol/L	30.1±4.3	0.3±0.3	***	
			˃45 nmol/L	0.6±0.6	46.1±4.2	***	
			Plasma folate [61] mean±SD	14.8±0.7	46.9±1.2	↑217%***	
			Prevalence of folate intake less than EAR in WRA [61]	79±4	13±1	***	

Table 4 (continued)

Country (study coverage)	Fortified foods (folic acid amount)	Mode of fortification (year)	Observed outcomes [ref]	Pre-fortification	Post-fortification	Relative Risk (95%CI); observed change	Type of study, data source and observations
Multiple LMIC	Wheat flour	Mandatory Mean duration of 4.2 y (range:1–11)	NTD prevalence [51]:	Period: by study	Period: by study		Systematic review and meta-analysis including 17 studies that evaluated the food fortification programs (16 national programs) from LMIC, mostly from Central and South America, Asia, and Africa. Note: Prevalence numbers calculated according to the author's data of NTD cases and births (cases/births per 10,000.)
			Total (8 studies)	9.2 per 10,000	5.8 per 10,000	0.59 (0.49–0.70); ↓41%	
			SB (9 studies)	4.1 per 10,000	2.6 per 10,000	0.66 (0.53–0.82)	
			Anencephaly (9 studies)	4.7 per 10,000	2.6 per 10,000	0.49 (0.40–0.60)	
			Encephalocele (8 studies)	1.1 per 10,000	0.80 per 10,000	0.64 (0.47–0.88)	
			Folate deficiency in WRA, (1 study)	17.8	3.1	0.20 (0.15–0.25)	
High-income countries							
United States (National)	Wheat flour (1.4 mg/kg)	Mandatory (1998)	NTD prevalence [63] (SB + Anencephaly)	Period: 1995–1996 3.8 per 10,000	Period: 1998–1999 3.1 per 10,000	0.81 (0.75–0.87); ↓19%**	Uncontrolled, before-and-after series. National study of birth certificate data for live births in 45 US states and Washington.
			NTD prevalence [64] (SB + anencephaly):	Period: 1995–1996	Period: 1999–2011		Cross-sectional uncontrolled before and after series. Data from 19 population-based birth defects surveillance programs in the US from 1995-1996 and 1999-2011.
			Prenatal ascertainment	10.7 per 10,000	7.0 per 10,000	↓35%**	
			No prenatal ascertainment	6.7 per 10,000	5.3 per 10,000	↓21%	
			Folate levels [65]:	Period: 1988–1994	Period: 1999–2000		Cross-sectional uncontrolled before and after series. Data from two National Health and Nutrition Examination Surveys (NHANES): reflecting the time pre and post fortification.
			Mean of folate in RBC	380±5.5	603±15.1	↑59% **	
			RBC in women 20–39 y	341±6.2	556±16.1	↑63% **	
			Prevalence of Inadequate folate levels in RBC (<305 nmol/L)	39.1±1.3	3.7±0.6	↓95 **	

Table 4 (continued)

High-income countries							
Chile (Santiago)	Wheat flour (2.2 mg/kg)	Mandatory (2000)	NTD prevalence [66]	Period: 1999–2000	Period: 2001–2002		Uncontrolled, before-and-after series.
	Wheat flour update (1.8 mg/kg)	Mandatory (2009)		17.1 per 10,000	9.7 per 10,000	0.57(0.45–0.71); ↓43%**	Data from a hospital-based surveillance system for NTD live births and stillbirths in nine public hospitals from Santiago de Chile (25% of the population). Folate levels of 605 WRA before and after fortification
			Mean of folate levels [67]				
			Serum	9.7±4.3	37.2±9.5	**	
			RBC	290±10.2	707±179	**	
			NTD prevalence [68]	Period: 1999–2000	Period: 2001–2015		Uncontrolled, before-and-after series. Prospective hospital-based surveillance system for NTD live births and stillbirths in 11 public hospitals from Santiago de Chile.
				17.1 per 10,000	8.9 per 10,000	0.52 (0.45–0.61); ↓48%**	
Canada (regional)	Wheat flour or cornmeal (1.5 mg/kg) Pasta (2.0–2.7 mg/kg)	Mandatory (1998)	NTD prevalence [67]	Period: 1993–1997	Period: 2000–2002		Uncontrolled, before-and-after prevalence study. Livebirths, stillbirths, and termination of pregnancies in seven Canadian provinces.
				15.8 per 10,000	8.6 per 10,000	0.54 (0.49–0.60); ↓46% **	
			NTD prevalence [69]	Period: 1991–1997	Period: 1998–2001		Uncontrolled, before-and-after prevalence study. NTD data from Newfoundland and Labrador Clinical Genetic Program.
				43.6 per 10,000	9.6 per 10,000	0.22 (0.14–0.35); ↓78%**	
			RBC folate in WRA [69]	Period: 1991–1997	Period: 1998–2001		Folate levels from a survey of 437 non-pregnant women (age 19-44 y).
				625 mmol/L (95% CI:601–649)	818 mmol/L (CI95%:784–854)	**	

Table 4 (continued)

High-income countries							
Australia (national and regional)	Wheat flour for bread making (2–3 mg/kg) Voluntary (1996)	Mandatory (2009)	NTD prevalence [70]:	Period 1: 1980–1992 (P1, No intervention) Period 2: 1993–1995 (P2, Supplements)	Period: 1996-2006 (P3, Suppl + voluntary fortification)		Uncontrolled before and after series in Western Australia Province. NTD data from the population-based Western Australia Defects Registry, including live births, stillbirths, and pregnancy terminations diagnosed prenatally and up to 6 years of age.
			Anencephaly	P1: 8.6 per 10,000	P3: 5.9 per 10,000	0.68 (0.56–0.83)**; ↓32%	
				P2: 9.6 per 10,000			
				(PR=1.1 vs P1)*			Baseline period is P1. There was no significant prevalence difference between supplementation and no intervention in the pre-fortification period.
			SB	P1: 9.1 per 10,000	P3: 7.0 per 10,000	0.77(0.64–0.92)**; ↓23%	
				P2: 9.1 per 10,000			
				(PR=0.99 vs P1)*			
			Encephalocele	P1=1.7 per 10,000	P3: 1.2 per 10,000	0.66(0.42–1.02)*; ↓34%	Other studies comparing mandatory *vs.* voluntary fortification are commented on later in the manuscript and showed significant reductions in aboriginal and Torres Strait islanders' NTD prevalence only after mandatory fortification [71].
				P2=2.2 per 10,000			
				(PR=1.2 vs P1)*			
			Total NTD	Data not available	Data not available	0.7(0.61–0.79)**	
			Non aboriginal	Data not available	Data not available	0.9(0.61–1.32)*	
			Aboriginal				

NTD neural tube defects, *SB* spina bifida, *WRA* women of reproductive age, *EAR* estimated average requirement

*$p > 0.05$; **$p < 0.05$; *** $p < 0.001$

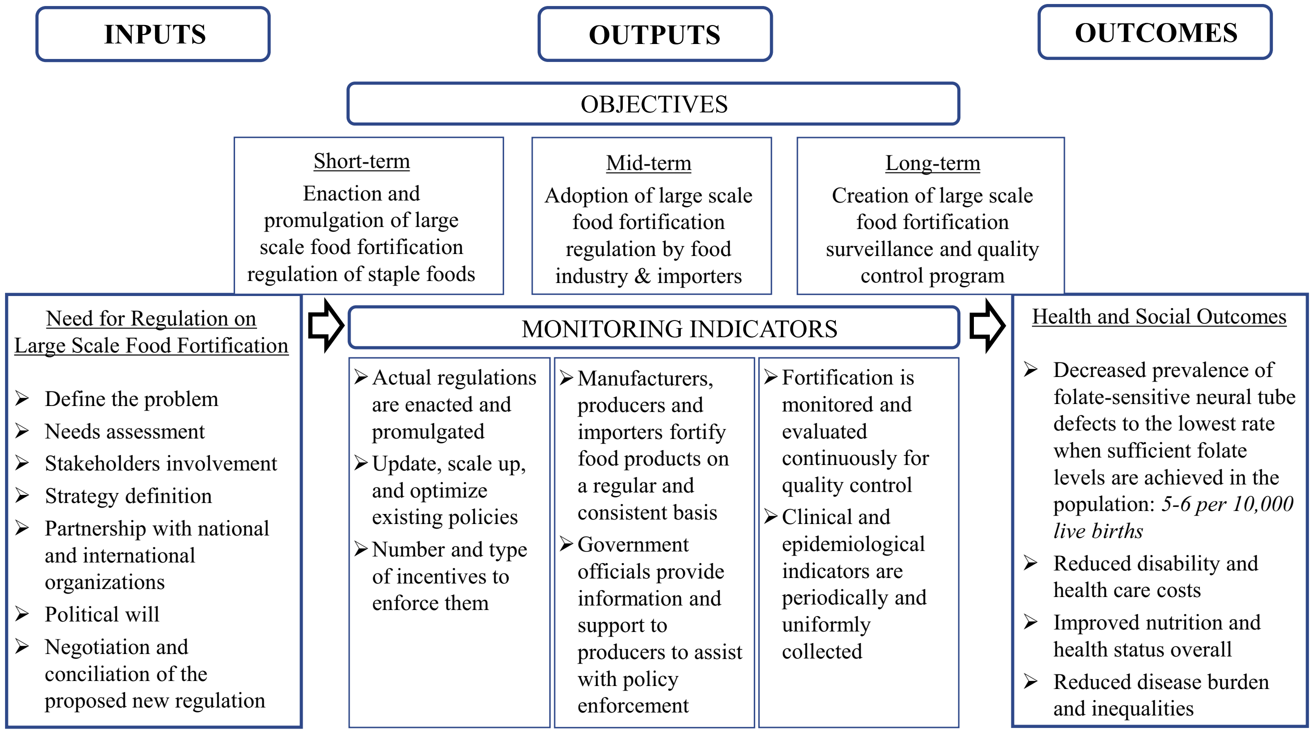


Fig. 2 A logic model for large-scale food fortification as an evidence-based policy to prevent neural tube defects (NTD). Source: authors' work using Microsoft's PowerPoint SmartArt

decision about the most appropriate food vehicle(s) to fortify [76]. Cultural and food habits are crucial factors in explaining the prevalence of folate insufficiency in different regions. Importantly, if the population has outliers due to ethnic origin or eating habits, this should also be considered in the sampling strategy [77].

Biological indicators such as serum and RBC folate help assess folate status (Table 2). The case study to measure folate status at the population level should have a representative number of WRA from different regions to evaluate the prevalence of folate insufficiency in the whole country. It is good practice to select a representative sample of rural and urban areas and, within them, select women from regions or localities with high and low NTD prevalence to serve as sentinel groups when testing the effectiveness of the intervention.

The first requirement for an appropriate vehicle for fortification is that it should be consumed by most of the population. Secondly, this food should be processed by a centralized industry (as opposed to being processed in multiple small entities, such as small mills in rural areas). Additionally, the added micronutrient(s) must not alter the accepted organoleptic characteristics of the food, including flavor, smell, taste, and, in most instances, color. Once added to food, the micronutrient should not be significantly affected by regular packaging and storage or traditional cooking practices. It is also critical that the price not be significantly impacted due to the fortification to assure vast consumption of the fortified food [50].

The most common staple foods used for LSFF with folic acid include cereals (wheat, corn (maize), and rice), but other fortified foods help improve folic acid delivery to the population, including milk and oil. There are, however, other foods and condiments that may constitute relevant alternatives for LSFF. A recent article has identified many foods that have been fortified with folic acid and have some evaluation, including retention of the micronutrient after production or storage, acceptability, and food and sensory characteristics after fortification. These foods include dairy products (low-fat milk, whole milk, powder milk, yogurt), condiments (salt, sugar, bouillon cubes), fruit juices, meat and eggs, fruit, other cereals (finger millet, teff), and candy [78]. Table salt and bouillon have attracted attention due to their potential to reach large segments of the population [79, 80].

A few countries have successful experience fortifying several foods simultaneously, like Costa Rica, which fortifies wheat flour, corn flour, milk, and rice, intending to reach different populations. This strategy has proved successful, as it is complementary and universal and has significantly reduced NTD prevalence, as different foods were added to the program in a stepwise manner [53].

Population reach and quality control are critical points in implementing any LSFF program. LSFF may be a voluntary practice (where food manufacturers add one or more micronutrients to their products, usually to increase profit but not regulated by the government) or a mandatory one (where the government mandates and regulates the fortification standards, sets up regulatory and monitoring practices and enforces them).

In settings of high coverage, poor functional results may be due to poor fortification quality and the absence of quality assurance/control programs that verify that food fortification meets the required standards. For example, one study from 12 countries with 20 national food fortification programs combined revealed that more than 50% of tested samples had micronutrients below the recommended levels [81]. An appropriate standardized legal framework for LSFF and well-established quality control mechanisms are needed for LSFF to effectively address the prevention goals at the population level [82].

Strategy definition, political will, approval, and legislation

Developing political will to fortify food with folic acid in LMIC is often challenging, as it requires efforts and funds to set up the fortification program. Given other priorities, allocating money to a food fortification program may be difficult [83]. However, mandatory LSFF is a government policy, so it is crucial to develop and ensure political will to move ahead with this policy. There is ample evidence to show that identifying one or more champions is a determining factor in increasing awareness and ensuring political will [83].

The next step is to build partnerships with stakeholders, including the food industry, universities, researchers, the public health sector (ministry of health), and the political sector, to help define the country-specific strategy. In LMIC, where resources are limited, collaborative work is crucial. The proposed fortification strategy should lead to the specific country promulgating a law appropriate to their context, including implementing, monitoring, and verifying the quality, safety, and efficacy criteria.

Legislation is the cornerstone of food fortification program implementation. It regulates all aspects of food fortification, including the list of foods to be fortified, micronutrients to be added, their level, and the program to control the efficiency. It regulates food manufacturing, packaging and labeling, quality assurance, and control of food production, storage, and transportation while promoting people's awareness and education. Legislation could be via state law or other governmental decisions. The local health ministry should prepare legislation with the other ministries and departments such as education, food production, transportation, and foreign affairs, among others. After adopting the law, food fortification becomes a mandatory process. However, it is necessary to allow a certain period so the industry may adapt to the regulation. After this, the process must remain under permanent supervision and strict control by the government. Monitoring and evaluation are essential to ensure industry compliance. Quality assurance procedures are needed to ensure fortification standards. Periodic surveys of folate levels will provide information about the effectiveness of the fortification program and may provide information to make any necessary corrections at the early stages of the process. Nationwide food-intake surveys will help amend the regulations most effectively.

A government dependency with a regulatory function such as a Food and Drug Administration will state that the codified policy or legislative regulations include situations and conditions in which the fortification of food with the nutrients listed is considered appropriate [84]:

- ✓ to correct a dietary insufficiency recognized by the scientific community to exist and known to result in nutrient deficiency disease or condition, such as NTD in the case of folate insufficiency
- ✓ to restore such nutrient(s) to a level(s) representative of the food prior to storage, handling, and processing
- ✓ in proportion to the total caloric content of the food, to balance the vitamin, mineral, and protein content
- ✓ to replace traditional food in the diet to avoid nutritional inferiority.

Public safety and awareness

Public safety is the highest priority when considering mandatory legislation. Safety in food fortification implies a reasonable certainty that the food additive is not harmful, although there is no absolute proof of the harmlessness of any substance in the present state of scientific knowledge. Safety can be determined by consideration of the following factors [85]:

- ✓ the estimated consumption of the additive with food based on its use
- ✓ the estimated cumulative effect of the additive on the diet
- ✓ the existence of safety factors generally recognized as appropriate

Nutrient mixtures added to food should be stable, physiologically available, present at a level that will not lead to excessive intake, suitable for fortification purposes (at the industrial level), and acceptable for food safety regulations. Food labeling cannot be false or misleading [86, 87].

The addition of different minerals and vitamins must be under strict control. Not every food is suitable for fortification with additives, depending on its chemical composition and potential interaction with food additives. Iodine, for example, is not stable and could quickly evaporate during food processing, such as cooking. Direct sunlight can also decrease the amount of iodine in salt. That is why the fortification of salt with iodine is going through the addition of potassium iodate, which is more stable and efficient in preventing iodine insufficiency. Folic acid is a more stable chemical agent applicable to frequently consumed foods such as flour and salt [87]. Also, the permissible level of micronutrients must be determined and constantly verified by monitoring programs.

Monitoring and evaluation

After enacting and implementing mandatory fortification, it is necessary to gather adequate evidence on the program's effectiveness, safety, and sustainability. This evidence is attainable through nutritional surveys, measurements of folate levels in WRA, and NTD prevalence through country-wide surveillance databases. Based on the experience of several LMICs and HIC that have successfully implemented food fortification with folic acid, the impact on NTD prevalence and folate levels became evident 2 to 3 years after their implementation [53, 59–64, 67–72]. After the first evaluation, the bottlenecks and weaknesses of the processes become evident and can be corrected.

In addition, it will be possible to identify the populations least and most benefited from the intervention and propose additional foods to fortify or complementary strategies which enhance the perinatal intake of folates in these populations. For example, in Costa Rica, universities and researchers, government entities in charge of public health, and the food industry have collaborated to generate evidence and strategies for continuous intervention improvement and maximizing resources.

The long-term sustainability of LSFF programs requires stakeholders' intervention at the system level, as illustrated in Fig. 3.

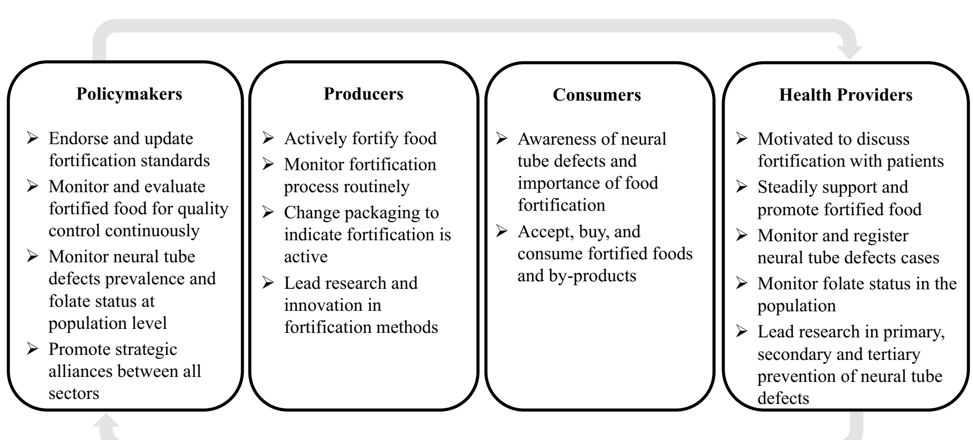
Building international collaboration

Convincing local government and medical officials to fortify a staple food or condiment with folic acid is a complex goal that requires coordinating multiple efforts. International professional and non-governmental organizations such as WHO, Nutrition International, Food Fortification Initiative, Global Alliance for Improved Nutrition, International Society for Pediatric Neurosurgery, and the Global Alliance for Prevention of Spina Bifida-F (GAPSBi-F) often play crucial advocacy roles [52, 88]. These organizations utilize available epidemiological and clinical data about NTD to convince local government officials on the urgency of LSFF with folic acid, using knowledge translation, transfer, and science diplomacy methods.

An international collaboration of local governments with WHO, UNICEF, medical institutions, and other organizations is essential in establishing high-quality standards for fortification programs. The local government can set up a liaison office with competent personnel to communicate with international organizations to provide logistic and scientific support. The involvement of local medical institutions will help get reliable NTD incidence and prevalence data, including an ETOPFA rate due to NTD, and evaluate the food fortification's success with folic acid through the follow-up programs. The leading local medical institution could serve as a collaborating center with the WHO that will encourage medical staff to improve public health through participation in joint research projects. Educational and research exchanges between physicians and medical researchers supported financially by WHO or other international organizations will serve this purpose.

An important aspect to consider, especially in LMIC, is the need for a reliable laboratory to conduct the recommended MBA to measure RBC folate. Given that the procedure requires a well-honed technique by adequately trained laboratory technicians, it is necessary to provide such training when conducting a national or subnational assessment. Furthermore, having an external reference laboratory checking for quality control samples is ideal. When folate surveys are not frequent in a given country, the trained laboratory may lose its expertise. To address these challenges, the Division of Laboratory Sciences of the US CDC has proposed a

Fig. 3 Actions needed for promoting sustainable system-level change on large-scale food fortification policies to prevent neural tube defects. Source: authors' work using Microsoft's PowerPoint



strategy to develop a global network of appropriately trained regional laboratories, which are certified, and periodically re-certified to conduct the folate MBA. These resource laboratories could work fee-for-service in different countries, ensuring quality results. By having different countries requiring these services, the reference laboratories will have a steady stream of work, thus maintaining their proficiency, ensured through the afore-mentioned re-certification program. Continued demand for their services would eventually lead to lower costs per sample. Furthermore, these laboratories could also help train other regional laboratories, expanding and strengthening the global network [89].

Likewise, international collaboration is vital in the implementation, standardization, and improvement of NTD surveillance systems in LMIC to have more reliable and genuine figures to characterize the NTD burden and assess the impact of the intervention.

Conclusion

Every year, thousands of infants and young children affected by NTD die or suffer permanent disability worldwide, and this condition disproportionately affects LMIC. However, this public health problem can be effectively prevented by implementing mandatory LSFF with folic acid. This intervention has improved folate levels in WRA in every low-middle- or high-income country that has enacted such a policy. This intervention is the most cost-effective way to improve micronutrient status in the population, providing a sufficient level of folate that can reduce NTD prevalence to 5–6 per 10,000 live births in every setting where the program is mandatory. Our review should encourage those countries which have not yet established an LSFF program to consider this intervention to reduce the burden of several micronutrient deficiencies, including nutritional anemia, iodine deficiency disorders, and NTD. LSFF can effectively reduce newborn, infant, and young child morbidity and mortality rates, thus improving the population's health, particularly in the most vulnerable segments.

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Author contribution All authors contributed to the study's conception and design. All authors contributed to the literature review and analysis. Kemel A. Ghotme conceptualized Figures 1, 2, 3. Adriana Benavides-Lara conceptualized and designed Table 4. Alexander Arynchyn, Kemel A. Ghotme, Mandana Arabi, and Homero Martinez performed critical revision of the manuscript. Homero Martinez wrote the first draft of the manuscript, and all authors commented on consequent versions. Kemel A. Ghotme conceptualized and devised the figures. All authors read and approved the final version of the manuscript.

Data availability All data provided in this article can be accessed through the corresponding references in the bibliography.

Declarations

Ethical approval This review paper dealt only with published material and did not include the participation of human subjects or animals. Therefore, the research was not submitted for review to a Human Subjects Protection Committee or Institutional Review Board. The authors state that the review of such published material was carried out with full respect and veracity of statements and facts and thus represents an authoritative assessment of the evidence reviewed.

Conflict of interest The authors declare no competing interests.

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