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Review Article

Impact of folic acid fortification of flour on neural tube defects: a systematic review

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Abstract

Objective: To review the impact of folic acid fortification of flour on the prevalence of neural tube defects (NTD).
Design: Systematic review of the literature on MEDLINE via PubMed, Scopus, OvidSP and LILACS (Latin American and Caribbean Health Sciences Literature) reporting the impact of folic acid fortification of flour on the prevalence of NTD in 2000–2011. Focusing on Santiago of Chile's birth defects registry (1999–2009) and the monitoring of flour fortification, we analysed the prevalence (NTD cases/10 000 births) pre and post flour fortification and the percentile distribution of folic acid content in flour (2005–2009). We explored the potential association between median folic acid in flour (mg/kg) and the prevalence of NTD.
Setting: Chile, Argentina, Brazil, Canada, Costa Rica, Iran, Jordan, South Africa and the USA.
Subjects: Live births and stillbirths.
Results: Twenty-seven studies that met inclusion criteria were evaluated. Costa Rica showed a significant reduction in NTD (~60 %). Prevalence in Chile decreased from 18·6 to 7·3/10 000 births from 1999 to 2007 and showed a slight increase to 8·5 in 2008–2009, possibly due to changes in fortification limits. When we related the prevalence of NTD with levels of flour fortification, the lowest prevalence was observed at a folic acid level of 1·5 mg/kg.
Conclusions: Fortification of flour with folic acid has had a major impact on NTD in all countries where this has been reported. Chile showed a 55 % reduction in NTD prevalence between 1999 and 2009. There is a need to constantly monitor the levels of flour fortification to maximize benefits and prevent the potential risk of folic acid excess, moreover to be vigilant for any new adverse effects associated with excess.

Keywords
Folic acid
Neural tube defects
Spinal dysraphism
Food fortification
Systematic review

The importance of nutrition in the aetiology of neural tube defects (NTD) was suggested by Hibbard and Smithells, who found that women deficient in folate had an increased number of abortions, placental abruptions and fetal malformations and intra-uterine growth retardation[1]. The first reports on folate deficiency and NTD, published by Smithells *et al.*[2,3], showed the protective effect of folic acid on the recurrence of NTD. A randomised study by Czeizel and Dudas[4] showed a decrease in first NTD occurrence in women supplemented with twelve vitamins containing 0·8 mg of folic acid, but not in women supplemented with trace elements and a very low dose of vitamin C. A rigorous double-blind randomised controlled trial supported by the UK Medical Research Council showed that supplementing women who had a history of children with NTD with 4 mg of folic acid daily decreased recurrence by 72 %[5]. Although some studies were retrospective, their results revealed an inverse relationship between NTD and folic acid consumption of 100–400 μg/d[6,7]. Based on this information, in 1992 the US Public Health Service recommended that all women planning to become pregnant consume 0·4 mg of folic acid/d starting one month prior to conception through the first trimester of pregnancy. Because supplementation not only reduced the occurrence of NTD but also their recurrence, it was recommended that women who already had an NTD-affected pregnancy

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consume 4 mg of folic acid/d throughout the pregnancy[8]. In 1998, the US Food and Drug Administration mandated that flour be fortified with folic acid to ensure an adequate supply of folate for women of childbearing age[9]; currently, about sixty countries have similar mandates to ensure adequate access to folic acid[10].

Unlike studies that evaluate the effect of folic acid supplementation on the occurrence NTD[11–13], we conducted a systematic review of uncontrolled studies in different countries to assess the effectiveness of national folate fortification programmes at preventing birth malformations, the NTD prevalence trends in Chile before and during fortification (1999–2009) and the potential relationship between Chilean NTD and the folic acid content of wheat flour.

Materials and methods

The systemic review included English and Spanish scientific journals between January 2000 and December 2011. The databases used included the following: MEDLINE/PubMed, Scopus, OvidSP and Latin American and Caribbean Health Sciences Literature (LILACS). The descriptors used to search 'Medical Subject Headings' (MESH) included the following: neural tube defects, spinal dysraphism, anencephaly, combined with folic acid and prevalence. From the 1185 articles identified, 106 potential studies were retrieved for detailed assessment after discarding duplicate articles, reviews and certain study designs. The articles were read and evaluated by one reviewer and checked by a second one using the inclusion and exclusion criteria (Table 1). Studies that reported exclusively non-NTD malformations were excluded, as were studies that assessed changes in the NTD prevalence in response to folic acid in combination with other vitamins. Based on the application of these criteria, twenty-seven studies of changes in the prevalence of NTD, spina bifida and anencephaly in response to mandatory folic acid food fortification were included in the present review (Fig. 1).

Annual NTD prevalence in Chile was estimated from an Excel file database provided by the Epidemiology Department of the Chilean Ministry of Health. The database contained information from nine hospitals in Santiago, the capital of Chile, with established monitoring systems during the pre-fortification period (1999) and the fortification period (2000–2009). This prospective registry considered all newborns including live births and stillbirths, with a recorded birth weight greater than 500 g and/or audited as fetal deaths, deaths under 1 year of age, hospital discharge birth registry books, newborn records, autopsies and/or other medical records. All NTD, whether associated with other malformations, were listed and categorised based on the severity of the defects.

Information on the folic acid content of wheat flour in Chile was requested via email from the General Director of the National Public Health Institute of Chile. The folic acid (mg/kg) content, determined by liquid chromatography, of the samples collected each trimester from the wheat flour mills registered with the National Public Health Institute in 2005–2009 was shared electronically as an Excel spreadsheet[14]. The STATA statistical software package release 11 (2009; StataCorp LP) was used to calculate the annual percentile distribution of the folic acid content in the flour samples and the annual prevalence of NTD. NTD prevalence was calculated as (total number of children with NTD/total number of newborns) $\times$ 10 000. We calculated the standard error of annual NTD prevalence based on the annual prevalence for each hospital included in the surveillance system. Descriptive analyses were used to describe the relationship between the annual NTD prevalence and the median level of folic acid in wheat flour (mg/kg) considering the concurrent year.

Table 1 Inclusion and exclusion criteria

Inclusion criteria
Uncontrolled studies that describe NTD, spina bifida and anencephaly prevalence in response to mandatory acid folic food fortification
Exclusion criteria
Summaries, case reports, editorials, reviews
Case–control studies, cohort studies and randomised clinical trials
Studies that describe response to folic acid in other congenital malformations
Association between NTD and folic acid supplements
Associations between other nutrients and NTD

NTD, neural tube defects.

Results

Of the twenty-seven studies reported in the present systematic review[15–41], there was a drop in the prevalence of NTD in fifteen[15–29] of them in response to fortification (Table 2). The largest drops were observed in Costa Rica (58 %)[28] Argentina (49·7 %)[25] and Canada (49 %)[21]. The smallest decrease occurred in the state of California in the USA (15·5 %)[23]. Twenty-one articles reported on the prevalence of spina bifida before and after flour fortification[15,16,19,21–23,25,26,28,30–41]. The largest reductions reported were in Costa Rica (61 %)[28], Canada (55 %)[21] and Chile (55 %)[25] The latter study examined the number of NTD cases between the years 1998 and 2007. The smallest reduction in the prevalence of spina bifida was in the USA (3·4 %)[40] This last study looked at two post-fortification periods (1999–2000/2003–2004) and assessed the effect of fortification stratified by ethnicity. The greatest decline in spina bifida in the USA was in Hispanics, who also had the highest prevalence of spina bifida before fortification. The smallest decline was in blacks, who also had the lowest prevalence of spina bifida before fortification[36].

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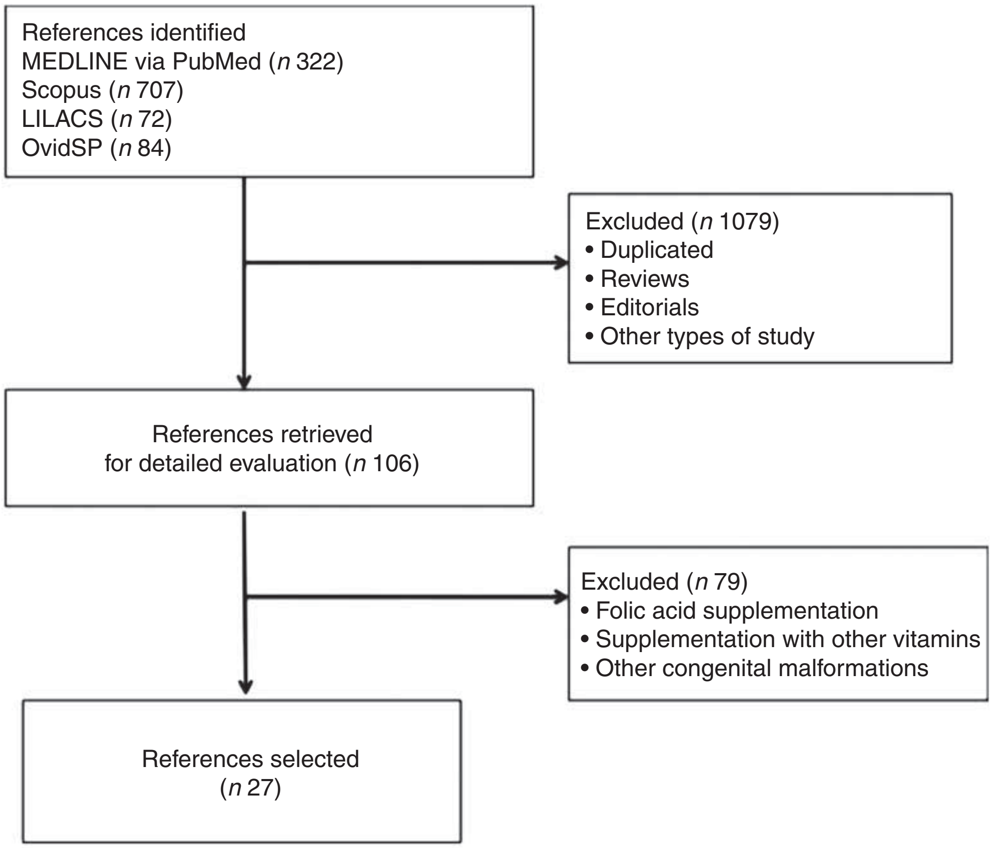

Fig. 1 Flowchart showing selection of articles for the current review (LILACS, Latin American and Caribbean Health Sciences Literature)

With respect to anencephaly[15,16,19,21–23,25,26,29,30,32–37,39,40], the greatest reductions after fortification observed were in Costa Rica (68 %)[28] the province of Ontario in Canada (58 %)[16] Argentina (57 %)[25] and Chile (50 %)[35]. The smallest reduction was in South Africa (9·8 %)[22] and in African Americans in the USA (9·1 %)[36]. Data presented for the state of Arkansas in the USA showed no reduction in the prevalence of anencephaly after fortification[19].

The annual NTD prevalence provided by the Chilean surveillance system for nine Santiago hospitals during the pre- (1999) and post-fortification (2000–2009) years is shown in Fig. 2. One year after fortification began (2001) there was a significant reduction in NTD prevalence (42 %). The lowest prevalence was reached 7 years after fortification began (7·03/10 000 newborns), a 60 % reduction from the number of cases in 1999. Overall, the decrease in NTD between 1999 and 2009 was 55 %. There was a slight increase in NTD (~20 %) in 2008–2009 compared with 2007, the year with the lowest prevalence in the period analysed.

Table 3 shows the percentile distribution of folic acid content of flour (mg/kg) between the years 2005 and 2009 in Chile. In 2005, the median folic acid content reached 1·9 mg/kg, close to the limit established by Chilean norms (2·2 mg/kg)[42]. It is important to mention that at least 10 % of the flour samples analysed from 2005 to 2009 contained non-detectable amounts of folic acid. On the other hand, 20 % of the flour samples contained a concentration in excess of that established by the norm. In 2008, there was a significant decrease in the folic acid levels in comparison to previous years (median = 1·1 mg/kg), increasing in 2009 (median = 1·6 mg/kg), when at least 5 % of the samples had folic acid concentrations greater than 5·4 mg/kg.

The time course of the association between NDT prevalence and median wheat flour folic acid content (mg/kg) in the concurrent year is shown in Fig. 3. The highest prevalence of NTD during the period studied occurred when wheat flour folic acid content was at its lowest (median = 1·1 mg/kg) and the lowest prevalence occurred when median folic acid reached 1·5 mg/kg.

Discussion

NTD are birth defects generated during the very early stages of embryonic development; they have a major impact on the health and quality of life of affected children and their families. Mandatory food fortification with folic acid has proved to be a cost-effective way to provide this critical nutrient during the periconceptional period and reduce the number of children affected by NTD[22,43–45].

Table 2 Description of the studies assessing the impact of flour fortification with folic acid on the prevalence of NTD

First author, year of publication, reference	Country	Period	Spina bifida*			Anencephaly*			NTD*			Source of data
			PreF	PostF	%	PreF	PostF	%	PreF	PostF	%	
Honein, 2001[15]	USA	1995–1996/ 1998/1999	2·62	2·02	22·0	1·16	1·03	11·2	3·78	3·1	18·0	Birth certificates from forty-five states and DC (CT, MD, NM, NY and OK were excluded)
Mathews, 2002[30]	USA	1996/2001	2·63	2·00	24·0	1·19	0·94	21·0				Birth certificates from State Vital Statistics Office (MD, NM and NY were excluded)
Ray, 2002[16]	Canada, ON	1994–1997/ 1998–2000	7·5	4·2	44·0	3·8	1·6	57·9	11·3	5·8	48·0	Antenatal diagnosis of NTD on ultrasonography or fetal autopsy after therapeutic termination and all live births and stillborns affected (>20 weeks' gestation)
Meyer, 2002[31]	USA, NC	1995–1996/ 1998–1999	6·46	4·70	27·0							Live births and the stillborns infants >20 weeks' gestation from Birth Defects Monitoring Program Collecting Information
Williams, 2002[32]	USA	1995–1996/ 1998–1999	6·68	4·04	39·5	4·18	3·36	19·6				Live births, fetal deaths and elective pregnancy terminations in twenty-four states (nine states ascertained prenatally diagnosed NTD)
De Wals, 2003[17]	Canada, QC	1996/2000							19·8	13·0	34·0	Live births and elective terminations of fetal malformations reported in the hospital administrative database (MedEcho)
Chen, 2004[18]	Costa Rica	1996–1998/ 1999–2000							9·7	6·3	35·0	Register of live births and stillborns weighing >500 g from twenty-four public hospitals
Mersereau, 2004[33]	USA	1995–1996/ 1999–2000	6·4	4·1	40·0	4·2	3·5	16·7				Live births, deaths stillbirths, fetal deaths and elective terminations from eight population-based birth defects surveillance systems
Simmons, 2004[19]	USA, AK	1993–1995/ 1999–2000	7·8	4·4	43·6	3·8	3·8	0	10·9	8·2	24·5	Prenatal and postnatal diagnosis cases (≤2 years old) from the Arkansas Reproductive Health Monitoring System including live births, stillbirths, elective terminations and spontaneous abortions

Table 2 *Continued*

First author, year of publication, reference	Country	Period	Spina bifida*			Anencephaly*			NTD*			Source of data
			PreF	PostF	%	PreF	PostF	%	PreF	PostF	%	
Hertrampf, (2004)[20]	Chile	1999–2000/ 2001–2002							17·0	10·1	40·5	Birth defects surveillance system in nine hospitals of Santiago City including live births and stillbirths >500 g
Canfield, 2005[34]	USA	1995–1996/ 1999–2000	4·9	3·2	34·7	2·2	1·8	44·0				Population-based data from twenty-three states (eight ascertain birth defects among pregnancy terminations) and surveillance methods to identify prenatally diagnosed and elective termination cases
López-Camelo, 2005[35]	Chile	1990–2000/ 2001–2002	9·33	4·77	46·7	6·39	3·18	50·2				Latin American Collaborative Study of Congenital Malformations in Chileans hospitals including live births and stillbirths weighing >500 g (approximately ≥22 gestational weeks)
Williams, 2005[36]	USA	1995–1996/ 1998–2002	H: 6·49 W-NH: 5·13 B: 3·57	H: 4·18 W-NH: 3·37 B: 2·90	H: 35·6 W-NH: 34·3 B: 18·8	H: 3·85 W-NH: 2·79 B: 1·98	H: 2·84 W-NH: 1·98 B: 1·80	H: 26·2 W-NH: 29·0 B: 9·1				Data from twenty-one birth defects surveillance systems (nine ascertained prenatally diagnosed NTD cases)
Besser, 2007[37]	USA, Atlanta	1982–1993/ 1994–2003	4·7	3·5	25·5	2·9	2·5	13·8				Data from the Program Metropolitan Atlanta Congenital Defects including infants and fetuses of ~20 weeks' gestation (hospital logs, disease indices, report of genetic tests and birth and fetal death certificates)
De Wals, 2007[21]	Canada	1995/2002	9·1	4·1	54·9	5·6	3·4	39·3	16·9	8·6	49·1	Study population including live births, stillbirths and terminations of pregnancy among women residing in seven Canadian provinces
De Wals, 2008[38]	Canada	1993/2002	8·6	4·0	53·5							Study population including live births, stillbirths and terminations of pregnancy among women residing in seven Canadian provinces
Sayed, 2008[22]	South Africa	2003–2004/ 2004–2005	9·3	5·4	41·9	4·1	3·7	9·8	14·1	9·8	30·5	Systems surveillance from twelve public hospitals (four provinces) including live births and stillbirths
Calvo, 2008[39]	Argentina	2000/2005	24·25	13·20	45·6	4·08	1·89	53·7				Hospital discharges of children (under 1 year old) from public hospitals

Table 2 *Continued*

			Spina bifida*			Anencephaly*			NTD*			
First author, year of publication, reference	Country	Period	PreF	PostF	%	PreF	PostF	%	PreF	PostF	%	Source of data
Boulet, 2008[40]	USA	1999–2000/ 2003–2004	3·51	3·39	3·4	2·47	1·98	19·8				Birth certificates and prenatal data of live births and stillbirths from twenty-one birth defects surveillance systems
Chen, 2008[23]	USA, CA	1989–1996/ 1998–2003	5·49	4·55	17·1	3·47	2·66	23·3	8·52	7·20	15·5	Live births, fetal deaths (≥20 weeks' gestation, fetuses spontaneously aborted (<20 weeks' gestation) from eight California counties
Pacheco, 2009[24]	Brazil, Recife	2000–2004/ 2005–2006							7·2	5·1	29·2	National Information System on live births
López-Camelo, 2010[25]		1998–2001/										Latin American Collaborative Study of Congenital Malformations (17, 41 and 19 hospitals in Chile, Argentina and Brazil, respectively) including live births and stillbirths >500 g
	Chile	2005/2007	10·2	4·6	54·9	6·3	3·7	41·3	19·8	10·1	48·9	
	Argentina		12·7	6·6	48·0	8·6	3·7	57·0	24·5	12·3	49·7	
	Brazil		14·5	14·2	2·1	11·2	6·9	38·3	31·4	24·3	22·6	
Amarin, 2010[27]	Jordan, North Area	2000–2001/ 2005–2006							18·5	9·5	48·6	Live births (Princess-Badea Teaching Hospital)
Collins, 2011[26]	USA, SC	1996–1997/ 2008–2009	6·1	4·3	29·5	5·9	3·9	33·8	13·4	9·7	27·6	Results of maternal α-fetoprotein laboratories, amniocentesis testing, pregnancy ultrasound scanning programme, medical reports from delivery hospitals, birth and death certificates
Orioli, 2011[41]	Brazil	2004/2006	23·1	14·0	39·3							Live Births Information System
Barboza, 2011[28]	Costa Rica	1997/2009	7·3	2·9	61·0	3·7	1·2	68·0	12·0	5·1	58·0	Congenital Disease Registry Centre
Abdollahi, 2011[29]	Iran, Golestan	2006–2008							31·6	21·9	31·0	Live births and stillbirths (≥20 weeks' gestational age) and newborns weighing ≥500 g (Dezyani Hospital)

NTD, neural tube defects; PreF, pre-fortification period; PostF, post-fortification period; %, percentage reduction; H, Hispanic; W-NH, white non-Hispanic; B, black non-Hispanic.
*Prevalence per 10 000 births.

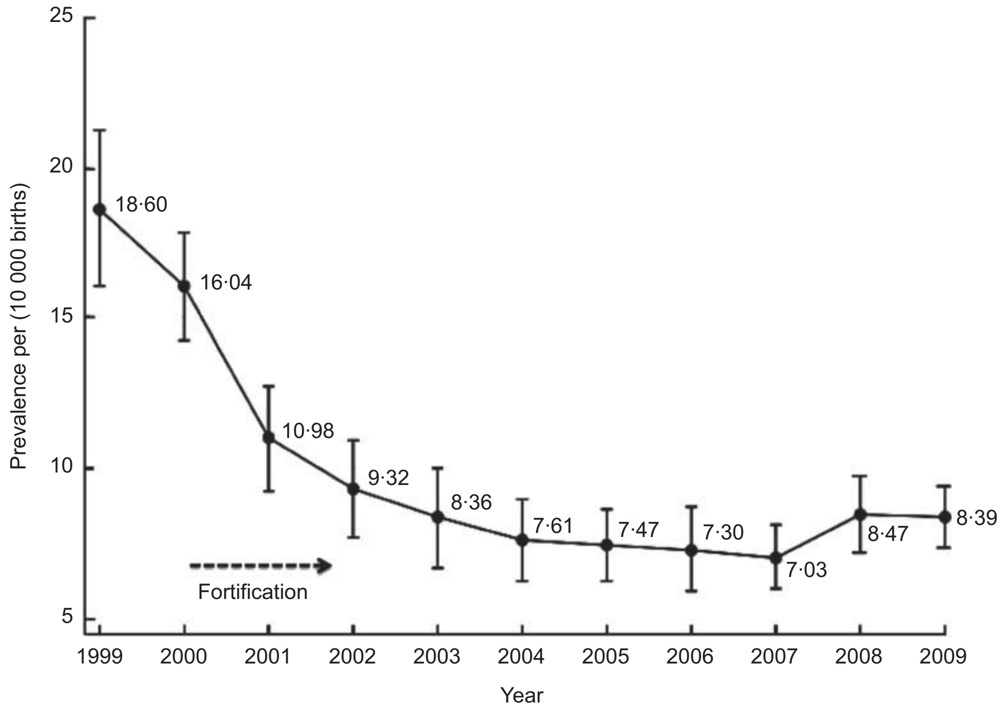

Fig. 2 Prevalence of neural tube defects in Chile, 1999–2009. Values are means with their standard errors represented by vertical bars

Table 3 Folic acid content in wheat flour (mg/kg) according to percentiles (P), Chile, 2005–2009

Year	*n*	P10	P20	P30	P40	P50	P60	P70	P80	P90	P95	P97
2005	338	0	0·59	1·21	1·51	1·90	2·20	2·50	3·00	4·30	5·90	7·40
2006	391	0·20	0·65	1·01	1·36	1·61	1·99	2·40	3·06	5·03	9·80	15·90
2007	279	0·10	0·58	0·99	1·27	1·51	1·77	2·30	2·80	4·80	8·60	10·10
2008	243	0	0	0·30	0·68	1·10	1·50	1·80	2·00	2·60	3·20	3·74
2009	287	0	0·67	1·02	1·26	1·58	1·92	2·27	2·78	3·68	5·40	7·70

Different mechanisms have been suggested to explain how folic acid might prevent NTD. Some authors have hypothesised that the presence of elevated folate receptor antibodies would limit folate transport to the early embryo, thus affecting its development[46]. Epigenetic mechanisms are likely involved in the aetiology. Given the role of folate in DNA methylation early during embryogenesis, lack of folate may affect neural tube closure and cause defects. Changes in DNA methylation that lead to over-expression of genes involved in autoimmunity[47] have been linked to the development of NTD. On the other hand, disruption of DNA methylation in animal models suggests that DNA methylation may also play a role in neural tube closure[47–49].

Mandatory folic acid flour fortification significantly increases serum folate levels and red blood cell count in women of childbearing age, helping prevent NTD[50,51]. The dose-dependent relationship between serum folate levels and the risk of having a child with an NTD has been described previously, with the highest risk occurring in women with significantly low folate levels in serum and red blood cells[52,53].

According to the current review, the largest reduction in NTD after food fortification was observed in Costa Rica. This country not only fortifies wheat flour but also maize flour, cow's milk and rice[28]. Other countries showed NTD reductions of about 50 %[16,20,21,57] and similar reductions in spina bifida[21,25,38] and anencephaly[16,35,39]. The results varied by country and depended on the integrity and quality of the country's surveillance system. For example, in some countries, the system is solely based on diagnostic information collected from birth certificates, which underestimates the number of stillbirths, fetal deaths and spontaneous and voluntary abortions[16]. Furthermore, the information collected prior to fortification may have been incomplete, thereby preventing accurate comparisons. Moreover, an adequate assessment of the impact of fortification on NTD may be compromised by women who take folic acid vitamins, which further increase their folate levels[17]. The decrease in NTD also varies by geographic region and social and demographic characteristics. Fortification has particularly benefited mothers with lower incomes and older women with no social security coverage[21,31]. The greatest impact of fortification has been

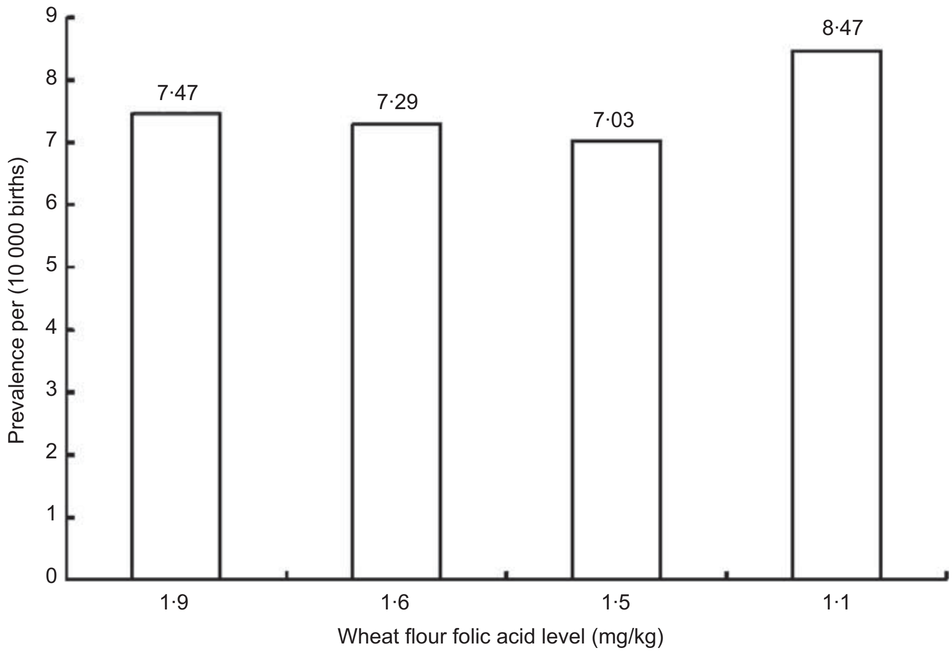

Fig. 3 Relationship between prevalence of neural tube defects in Chile and median folic acid content in wheat flour (mg/kg) in the concurrent year

observed in regions or countries with a higher prevalence of NTD prior to intervention[20,39,54]. Williams *et al.* looked at the prevalence of spina bifida and anencephaly in different ethnic groups in the USA. They found the largest reductions in the Hispanic population and the smallest reduction in the African-American population, which had a lower initial prevalence and, therefore, a lower potential reduction[32]. These differences must be considered in addition to food consumption patterns, which differ in intake of folate-rich foods and vitamin supplements.

The existence of polymorphisms in the genes encoding enzymes in the folate biosynthetic pathway, such as *MTHFR* 677CT and 1298AC, especially homozygous *MTHFR* 677TT, may account for differences in NTD prevalence considering the reduced activity of the enzyme methylenetetrahydrofolate reductase (MTHFR) that converts 5-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the main form of circulating folate[55,56]. Populations with a higher prevalence of these polymorphisms, such as the Hispanic population, have an increased number of congenital malformations compared with black or white populations. However, this increase in malformations does not occur in all groups with a high prevalence of these polymorphisms, suggesting that other genes or environmental factors may mediate this association. Other conditions, such as maternal diabetes and obesity, may also affect the presence of an NTD[57,58].

Some studies have shown that the decrease in the number of spina bifida cases is greater than the decrease in anencephaly cases[15,21,30,32,36,37]; however, other studies have shown a greater reduction in the latter[16,23,26,35,39,40]. The observed divergence in relation to the decrease in NTD is not well established. The smaller decrease in anencephaly cases is most likely the result of elective pregnancy termination being labelled as non-viable fetus, which may have also not been properly registered. Furthermore, there is no clear explanation for the higher number of female embryo anencephaly cases, which suggests that female embryos are more susceptible to environmental influences than male embryos. Alternatively, male embryos may be more susceptible to the lethal effects of toxin exposure, explaining the greater loss of male embryos with anencephaly in times of high prevalence[37].

When assessing the impact of flour fortification on NTD, it is important to acknowledge the secular trends of congenital malformations prior to the fortification of foods. However, this is not always possible because long-term data are needed and not always available.

Several countries in the Americas have implemented mandatory folic acid flour fortification, including Costa Rica, Guatemala, Honduras, Mexico, Nicaragua, Panama, Peru, Chile, Argentina, Canada and the USA; however, most of these countries do not have an adequate monitoring system for NTD and other malformations, which may limit the validity of the final evaluation[39].

Chile does not have a national surveillance system for NTD; however, it does have a system that has monitored congenital malformations in public and private hospitals over the past 30 years, a system which belongs to the Latin American Collaborative Study of Congenital Malformations (Estudio Colaborativo Latinoamericano de Malformaciones Congénitas, ECLAMC). This system monitors only approximately 7 % of total births in Chile, including live birth and stillbirth infants weighing over 500 g, which has permitted measurement of the secular trend as well as the impact of folic acid flour fortification in Chile[59].

According to data provided by López-Carmelo *et al.* of ECLAMC, Chile would not have had a downward trend in the number of NTD cases prior to the folic acid flour fortification. Therefore, the observed decrease in spina bifida and anencephaly cases in the first post-fortification period (2001–2002) can be exclusively explained by flour fortification[35], which caused a significant increase in folate in women of childbearing age[60]. In 1999, another surveillance system for registering NTD was established in nine public hospitals in Santiago, Chile's capital, accounting for 60 % of the city's births and 25 % of the country's[60]. In Chile, termination of pregnancies and therapeutic abortions are not permitted and almost all deliveries occur in institutionalised settings. Therefore, the underestimation of NTD is unlikely. The 55 % reduction in NTD between 1999 and 2009 demonstrates the positive impact of fortification in Chile. The present study's findings are based on the latest available data from the surveillance system of the nine hospitals mentioned previously. After 2009, the surveillance was interrupted (Fig. 2), limiting future assessments.

The mandatory folic acid flour fortification in Chile that began in 2000 (2·0–2·4 mg/kg) aimed to achieve a folic acid intake of approximately 400 μg/d[42]. However, national monitoring of folic acid in flour started in 2005 when an analytical technique was implemented at the Institute of Public Health[61]. The large dispersion observed in the levels of folic acid in flour samples (Table 2) forced the Ministry of Health in 2007 to request that milling companies adjust the amount of folic acid they add to wheat flour. This request resulted in a median decrease from 1·5 to 1·1 mg/kg in 2008, and a decrease from 8·5 to 3·2 mg/kg for samples located at the 95th percentile. While, on the one hand, the existence of samples without folic acid reflects problems of quality control in the milling industry that could negatively impact the prevention of NTD, high levels of folic acid, which were detected in approximately 20 % of the samples, could be a major risk factor in some populations. Although no data exist to ensure that the consumption of wheat flour-based food has remained stable among women of childbearing age, the described association between the annual NTD prevalence and annual median folic acid levels (median) suggests that a lower folic acid level can also be effective and that uncontrolled declines can negatively impact the results (Fig. 3).

It is important to keep in mind that since folic acid fortification started, there has been disagreement about what constitutes adequate levels of folic acid supplementation. To some extent these differences can be explained by the fact that the initial studies were designed to determine the role of folic acid in NTD, not the lowest dose at which benefit was achieved[62]. Daly *et al.* predicted a decrease in NTD prevalence of 22 %, 41 % and 47 % with folic acid consumption of 100, 200 and 400 μg/d, respectively[63], while Wald *et al.* predicted a decrease of 18 %, 35 % and 53 % with similar intakes[64]. In the studies that used multivitamins, the effect of folic acid could not be assessed independently, which is important given the recently described role of vitamin B_{12} in the prevention of these malformations[65]. Although the association between serum folate and NTD at low levels is rather proportional than linear, the effects of high levels of folate intake have not been clearly established. Despite Wald *et al.*'s description of a linear (logarithmic) relationship among folic acid intake, serum folate and NTD reduction, with estimates that go beyond the main data, it is more common to observe a stabilisation that follows a saturation pattern, as observed in many metabolic processes, or a U-shaped curve with an increasing prevalence at the upper end[66]. From the serum folate values obtained in a variety of studies[63,67–69], some have estimated that adequate NTD prevention can be obtained with intakes close to 100 μg of folic acid/d, especially if the food is fortified and its consumption is constant and prolonged[63,66]. This adaptation of the folic acid recommendation would maintain the benefits, while limiting the exposure of other population groups that do not necessarily benefit from folic acid food fortification[70–72], with the possibility of recommending higher doses in special cases such as women with a positive history of children with NTD or polymorphisms associated with folate metabolism[73]. These adjustments should be kept in mind because some animal and clinical studies suggest that folate possesses dual modulatory effects on colorectal cancer development and progression, depending on the timing and dose of folic acid intervention[70,74–76]. Moreover, other studies have described positive associations between high serum folate and both anaemia and poor cognitive test performance in persons deficient in vitamin B_{12}[71,77].

Conclusions

The studies included in the current review show that fortification of flour with folic acid has significantly reduced the number of children with NTD in all countries that have mandated it. In Chile, the mandatory fortification of wheat flour has led to a significant reduction in NTD. One of the limitations for its supervision and evaluation has been the delayed implementation of a system for monitoring flour folic acid content and the lack of an adequate national surveillance system for congenital malformations. This situation shows the importance of establishing appropriate quality controls and a continuous monitoring system from the start, which in turn allows for early adjustments to fortification levels to better achieve the desired goal of NTD prevention while avoiding the potential consequences of excess.

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