Folic Acid Fortification of Wheat Flour: Chile

Eva Hertrampf, M.D., and Fanny Cortés, M.D.

Neural tube defects (open spina bifida, anencephaly, and encephalocele) represent the first congenital malformations to be preventable through public health measures such as supplementation and/or food fortification with folic acid. In Chile, starting in January 2000, the Chilean Ministry of Health legislated to add folic acid to wheat flour (2.2 mg/kg) to reduce the risk of NTDs. This policy resulted in an estimated mean additional supply of 427 μg/d in significant increases in serum folate and red cell folate of 3.8 and 2.4-fold, respectively, in women of fertile age, one year after fortification. The impact on the rate of NTDs is presently being studied in all births, both live births and still births, with birth weight >500 g in the city of Santiago. Preliminary results show a reduction of 40% in the rates on NTDs from the pre-fortification period (1999–2000) to post-fortification period (2001–June 2002). Fortification of wheat flour with folic acid in Chile is effective in preventing NTDs in Chile.

Key words: neural tube defects, folic acid

doi: 10.1301/nr.2004.jun.S44–S48

Background

Infant mortality in Chile is 8.9/1000. Congenital malformations are the second cause of infant mortality in Chile, after prematurity.[1] Neural tube defects (NTD) resulting in congenital malformation of the nervous system—such as anencephaly and spina bifida—are the second most significant single malformation after congenital heart diseases. These malformations lead to stillbirth, death in early infancy or a lifetime of disability. In Chile, the incidence corresponds to 1.7/1000 live births according to ECLAMC (Spanish acronym for Estudio Colaborativo Latinoamericano de Malformaciones Congénitas) registry and rates have not changed between 1967 and 1999.[2]

According to this NTD incidence rate, an estimate of four hundred babies affected with NTD are born every year. In Chile, pregnancies cannot be terminated and even therapeutic abortion is not permitted by law. Factors such as the high cost of lifetime medical attention of a patient with spina bifida and the incalculable emotional cost on the family make NTD a major public health problem in the country. A peri-conceptional increase in the intake of folic acid has shown to reduce the prevalence of NTD.[3,4] Food fortification with folic acid as the intervention seems likely to succeed in increasing folate intake among populations throughout the world, especially in less-developed communities. Dietary changes and prophylactic supplementation appear as unfeasible strategies in these environments, due to the lack of means for educational campaigns, lack of compliance, and lack of access, information and services for peri-conceptional folic acid supplementation, and the fact that most pregnancies are not planned.

Description of Fortification Initiative

In Chile, a group of academics and program planners from the Ministry of Health—including people from the local mill industry—identified the folic acid fortification of wheat flour as a promising strategy for increasing folic acid intake in the population based on several reasons: (a) wheat flour is a staple food in the whole country; (b) milling for bread-making corresponds to 90% of the total, besides, more than 70% of wheat flour is used for making the type of bread typically consumed by Chileans[5] (80 g of wheat flour/100 g of bread) known as *marraquetas and hallullas;* (c) mills are technologically developed and already have quality assurance systems in place; these features have permitted the successful process of wheat flour fortification with iron as ferrous sulfate (30 mg/kg), thiamine (6.3 mg/kg), riboflavin (1.3 mg/kg), and niacin (13.0 mg/kg) since the year 1951[6]; (d) mean intake of wheat flour as bread in Chile is very high, approximately 200 g/day[7]; (e) monitoring of quality assurance is permanently being conducted at the pre-mix vendors and mills level by the Institute of Public Health; (f) the cost of adding folic acid to the pre-mix is low (approximately US$ 0.15/ton of wheat flour) so it can be absorbed by the milling industry[8]; (g) total cost of rehabilitation for a child affected with spina bifida in

Drs. Hertrampf and Cortés are with the Institute of Nutrition and Food Technology, (INTA), University of Chile, Casilla 138-11, Santiago, Chile.

Chile was estimated as US$ 120,000 (from birth to 18 years of age) and the cost of adding folic acid in US$ 175,000/year.[8] Hence, two cases of NTD prevented in a year would permit the recovery of the fortification cost with folic acid (FA) for a whole year.

Starting in January 2000, the Chilean Ministry of Health mandated a regulation requiring that FA be added at a level of 2.2 mg/kg to the pre-mix currently in use for wheat flour. This policy, tailored on bread consumption by the target group, was expected to result in a mean additional intake of approximately 400 μg/day in women of childbearing age (15 to 44 years). It is important to note that folic acid-fortified foods such as breakfast cereals are scarce and economically out-of-reach for most of the population. Also, there is very little, if any, consumption of folic acid supplements. Therefore, bread fortified with folic acid would be the main source of this nutrient benefiting the entire population.

Impact Evaluation of Folic Acid Flour Fortification Program in Chile

The situation described above provided a great opportunity to assess the effectiveness of the FA flour fortification program in the reduction of the risk of NTD. These results could be used as a case study to sustain and improve actions for other countries in the region. Currently, reported information on the effect of FA fortification of foods originates from the United States[9] and Canada.[10]

The evaluation of the Chilean intervention was undertaken by a group of researchers at INTA, University of Chile, sponsored by Pan-American Health Organization/World Health Organization and financed by the March of Dimes, Centers for Disease Control, United States and the Chilean Ministry of Health, with the collaboration of the University of Florida. The assessment of the effectiveness of the FA flour fortification program was reviewed before and after implementation, and included: (a) increasing bread folate content, improving folate status in women of childbearing age, and (b) reducing the frequency of NTD in the population.

Folic Acid Content of Bread

The Institute of Public Health (Ministry of Health) monitors iron and B vitamins, but not folic acid in pre-mix and wheat flour. To measure folic acid in bread, one kg of bread (*marraquetas* and *hallullas*) was purchased over the counter at 50 randomly selected bakery industries in the Metropolitan Area of Santiago. Samples were obtained three and six months after fortification started in the same bakeries. Folate was extracted from the sample using a modification of the tri-enzyme extraction method.[11] Folate content was measured using the microplate adaptation of the microbiological assay at the University of Florida.[12] The folate content for the 100 bread samples was 202 $\pm$ 94 μg/100 g of bread (range 22–416 μg/100 g). Only 9/100 contained <37 μg FA per 100 g, suggesting they were made from unfortified flour. Distribution of the values confirmed that wheat flour was fortified four months after the law mandated date of FA fortification (Table 1).

Folic Acid Consumption from Bread and Changes in Blood Folates

Maternal Infant Health Program from the National Health Service covers at least 70% of Chilean population and is based on Outpatient Primary Care Clinics, which are located throughout the entire country. Mothers attend the Program for pregnancy and healthy baby care control. Seven hundred fifty-one women of low socioeconomic status of childbearing age with at least one child (and no family history of NTD) attending three outpatient clinics (Alejandro del Río, La Granja and La Faena) in Santiago, Chile were studied. Women were recruited and studied from October to December 1999 (before fortification), and assessed again in October to December 2000 (after fortification). A total of 605 women (81%) completed the follow up. Causes of loss to follow-up were moving out of reach (75), rejection of second venipuncture (65), death (2), incarceration (2). Subjects with both assessments and those lost to follow-up were similar with respect to bread consumption and blood folates. Other characteristics of the group included the following: (a) body mass index (26.4 kg/m^2 $\pm$ 5.1); (b) multiparous (2.2 $\pm$ 1.2 children); (c) 12.6% anemic (Hgb <12 g/dL); (d) 75% breastfed last child >6 months; (e) 23% used an oral contraceptive agent; and (f) ~60% did not smoke or consume alcohol. In this group of women (n = 605) average bread consumption was estimated based on a combination of a 24-hour recall and a food frequency questionnaire specifically designed to assess

Table 1. Folate Content in Bread from Bakeries in Santiago, Chile, 3 and 6 Months after the Mandate for Folic Acid Fortification

	Post-fortification Folate Content of Bread	
	April 2000	**August 2000**
Bakeries sampled (*n*)	50	50
Mean folate*	226.9	178.3
Median folate*	208.5	182.1
Range*	30.8–416.2	22.3–348.5
Samples < lower limit by law	11/50	17/50
Samples not fortified	2/50	7/50

*μg/100 g bread.

intake of bread and other wheat flour-based foods, FA-fortified foods, and vitamin supplements. Estimated FA intake was calculated based on bread consumption derived from the mean value of data obtained from both the 24-hour recalls and food frequency questionnaires and the mean bread folate content. The effect of fortification on blood folate concentration was evaluated in a follow-up study. Serum and RBC folate and vitamin B_{12} concentrations were analyzed using Bio-Rad Laboratories (Hercules, CA, 1989) QuantaPhase II Folate Assay kit.

Folic Acid Consumption

The estimated median bread intake was 245 and 239 g/d before and after fortification. On a daily basis, 98% of the women consumed bread, and 89% ingested over 180 g/day. Of the bread eaten, 97% was industrially processed, corresponding to the type of bread typically consumed in Chile and analyzed. None of the subjects consumed other FA-fortified foods, and none took FA supplements. Mean FA intake was 427 (95% CI 409–445) g/day from estimates of the daily intake of FA from fortified bread based on reported consumption by the women studied. Almost half of them (48%) consumed >400 ± g of FA daily. Only 3% consumed <100 ± g/d. The intake of FA from bread for the rest of the group (49%) corresponded to values varying between 100 to 400 ± g/day.[13]

Changes in Blood Folates

The effect of habitual consumption of folic acid-fortified foods on folate levels is accepted as the best method for determining whether people are consuming more folic acid. Evaluation of the folate nutritional status in 605 women confirmed the improvement of folate intake, showing a remarkable increase of serum and red blood cell folate concentration after the program was implemented. Prior to fortification, the mean serum concentrations and red blood cell folate were 9.7 ± 4.3 and 290 ± 102 nmol/L, respectively, compared to 37.2 ± 9.5 and 707 ± 179 nmol/L, post-fortification ($P < 0.0001$). As expected, vitamin B_{12} concentrations did not change during this time (266 ± 105 and 268 ± 165 pmol/L). The distribution curves for serum and RBC folate concentrations before and after fortification show a striking shift to the right (A and B), in contrast to the vitamin B_{12} distribution curves, which were similar (C) (Figure 1).[13]

These findings demonstrate that regular consumption of a FA-fortified staple food is highly effective in improving folate status in women of childbearing age. Serum and RBC folate concentrations significantly increased after 10 months of consumption of FA-fortified wheat flour.

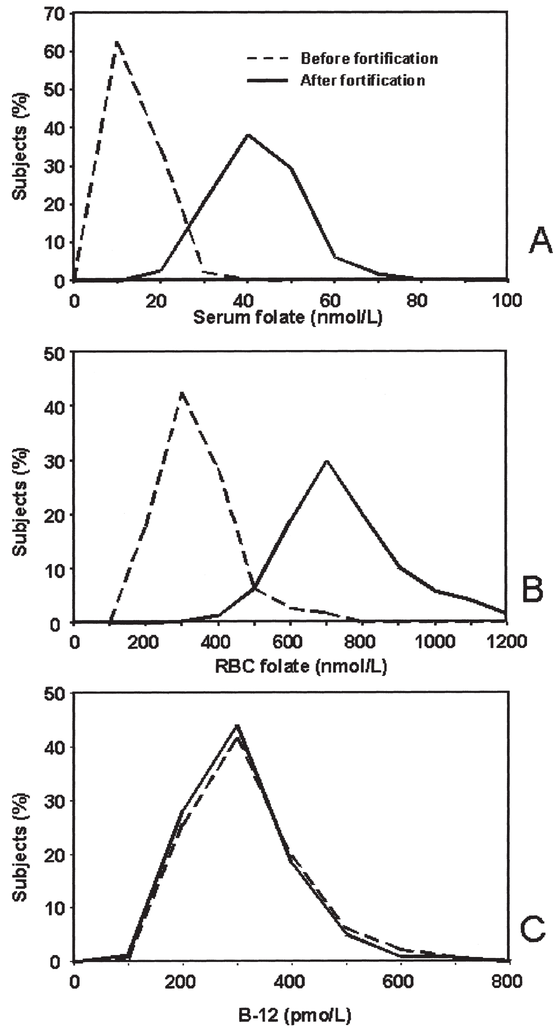

Figure 1. Serum folate, red cell folate, and vitamin B_{12} distribution curves before and after folic acid fortification of wheat flour in Chilean women of reproductive age in Santiago, Chile. From reference 13.

In Chile, the increase in blood folate concentration can be attributed to the consumption of FA fortified wheat flour. The study group did not consume other FA-fortified foods, such as breakfast cereals, because they are not culturally accepted, scarce, and economically out of reach. In addition, FA supplements were not taken by any of the study subjects since they have not been mandated or made available to this low-income population group by the Chilean public health service. Therefore, the wheat bread fortified with FA was the main source of this nutrient in the population studied.

INTA researchers began studying a group of Chilean elderly at the time that folic acid fortification first began; after six months of fortification, significant increases in serum folate levels were reported.[14] Since this age group is at a higher risk of vitamin B_{12} deficiency, increased intakes of FA provided by fortified wheat flour could be considered as an objection to mass food fortification for the risk of masking vitamin B_{12} deficiency, (deficiency without anemia because of folic acid) leading to irreversible neurological damage. Experts worldwide accept[15,16] that this was only likely to happen with folate intakes $>$ 1 mg/day. As yet, there has been no evidence that increased folic acid levels from fortification are harmful. Interestingly, in a recent study, a U.S. group of 1573 elderly showed no evidence of an increase in low vitamin B_{12} concentrations without anemia after fortification of cereals with folic acid.[17] In Chile, based on data suggesting that elderly population[18] and women of fertile age[13] are at risk of vitamin B_{12} deficiency, a suitable approach to this problem might be to introduce B_{12} fortification as well as folic acid fortification in wheat flour. It is important to note that folate deficiency is also widespread in the elderly,[18] so correcting hyperhomocysteinaemia through folic acid fortification might reduce considerable deaths from coronary heart disease and stroke.

Changes in NTD Frequency

Blood folates have been shown to correlate with folate consumption and appear to correlate with NTD rates as well.[19,20] Nevertheless, blood folate levels are still an intermediate outcome. The real measure of the impact of increased folic acid consumption is the reduction of NTD rates. To evaluate effectiveness, we decided to monitor the prevalence of NTDs to determine whether the rate declined after mandatory FA fortification.

In 1998 the only surveillance system in place for congenital malformations was ECLAMC.[2] ECLAMC reflected only 7% of total births in Chile, from three maternity hospitals, none of which included the public maternity hospitals in Santiago. Since birth certificates do not include information about malformations and the Chilean health system does not have a National Birth Defect Registry, a hospital-based surveillance system was established with assistance from the Centers for Disease Control (CDC) and ECLAMC to register NTDs. The system was established in nine public hospitals in Santiago in 1999, and registers all livebirth and stillbirth infants with birth weight ≥500 g. The number of births in these hospitals is approximately 60,000 per year, which accounts for 60% of births in Santiago and 25% in the country.

In Chile, 98.9% of deliveries occur in institutional settings, and 80% of them under the National Health Public System. About 40% of congenital malformations are diagnosed in prenatal controls; however, termination of pregnancies and therapeutic abortions are forbidden by law. A neonatal screening program for PKU and congenital hypothyroidism covers 98% of national births. Since the NTD registry is part of regular neonatal care, it is unlikely that NTDs, which are serious anomalies, will escape diagnosis. Newborns with spina bifida are discharged after they received surgery. Stillbirths must be audited and autopsies are mandatory in all cases. The above-mentioned features of this surveillance system provide concrete factual information based on monitoring all births. Furthermore, the potential for underestimating NTDs is not as likely, since termination of affected pregnancies and inadequate stillbirth registry methods are not included.

In each hospital one member of the staff (neonatologist or registered nurse) has been recruited and trained by the research team to revise all births, to register and to describe NTD. Types of NTD registered were anencephaly, encephalocele, and spina bifida associated or not with other malformations. If two neural tube defects in a newborn occur concomitantly, the anatomically higher defect is recorded. A specially trained clinical geneticist was hired to monitor the correct registration of NTD during the four years of registry. Data is obtained from a monthly review using the following sources: death under one year old audits, fetal death audits, hospital discharge report, book of deliveries, registry of newborn, registry of malformed newborns, registry of stillbirths, autopsy protocols and clinical records. Validation of the collected data is performed by the rest of the research team through the described sources periodically. Total prevalence rates are calculated as a total number of neural tube defects per 10,000 births.

Table 2. Total NTD Rates before Folic Acid Fortification* (January 1999 to December 2000) and after Fortification† (January 2001 to June 2002) in Santiago, Chile

	Before Fortification	After Fortification	RR (95% CI)	Decline %
NTD rates‡§	17.0	10.1	0.60 (046–0.77)	40

*120,636 births.
†88,538 births.
‡NTD births/10,000 births.
§Live and stillbirths.
NTD = neural tube defects, RR = relative risk, CI = confidence interval.

Folic acid fortification was mandated by January 2000; we verified compliance with fortification by April 2000 through assaying FA bread content. Thus, data were divided in two temporally defined groups: **Pre-fortification period,** data from January 1999 to December 2000, because neural tube development of the babies born in that period was not exposed to FA fortification, and **Post-fortification period,** data from January 2001 to December 2002.

Preliminary analysis of the data shown in table 2 revealed that the total NTD rate incidence, including live and stillbirths, decreased by 40% from the pre-fortification period (1999–2000) to post-fortification (2001–June 2002), from 17.2 to 10.1 per 10,000 births (RR 0.60, 95% CI 0.46–0.77).

This decline is temporarily associated with the FA fortification of wheat flour. Increases in FA intake and blood folate concentrations by women of reproductive age after FA fortification strengthen the conclusion that this intervention is effective in preventing NTD in Chile.

1. Ministerio de Salud de Chile. Estadísticas de mortalidad y natalidad, Chile. 2000. http://epi.minsal.cl/epi/html/frames/framel/htm, March 2003.
2. Nazer J, Lopez-Camelo J, Castilla E. ECLAMC: results of thirty years of epidemiological surveillance of neural tube defects. *Rev Med Chile.* 2001;129: 531–539.
3. Czeizel AE, Dudas I. Prevention of the first occurrence of neural tube defects. *N Engl J Med.* 1992; 327:1832–1835.
4. MRC Vitamin Study Research Group. Prevention of neural tube defects. *Lancet.* 1991;338:131–137.
5. Instituto Nacional de Estadística (INE), Banco Central, Servicio Nacional del Consumidor. Chile, 1997.
6. Hertrampf E. Iron fortification in the Americas. *Nutr Rev.* 2002;60:S22–S25.
7. Castillo C, Atalah E, Benavides X, Urteaga C. Patrones alimentarios en población adulta de la Región metropolitana. *Rev Med Chil.* 1997;125:283–289.
8. Ministerio de Salud de Chile. Norma técnica para la fortificación de la harina de trigo con vitaminas y minerales. Diciembre 1999.
9. Honein MA, Paulossi LJ, Mathews TJ, Erickson JD, Wong LYC. Impact of folic acid fortification of the US food supply on the occurrence of neural tube defects. *JAMA.* 2001;285:2981–2986.
10. Persad VL, Van den Hof MC, Dube JM, Zimmer, P. Incidence of open neural tube defects in Nova Scotia after folic acid fortification. *CMAJ.* 2002;167: 241–245.
11. Martin JI, Landen WO Jr, Soliman AG, Eitenmiller RR. Application of a tri-enzyme extraction for total folate determination in foods. *J Assoc Off Anal Chem.* 1990;73:805–808.
12. Tamura T. Microbiological assay of folates. In: Picciano MF, Stokstad ELR, Gregory JF III, eds. *Folic Acid Metabolism in Health and Disease. Contemporary Issues in Clinical Nutrition.* New York: Wiley-Liss; 1990:121–137.
13. Hertrampf E, Cortés F, Erickson D, et al. Consumption of folic acid-fortified bread is highly effective in improve folate status in women of reproductive age in Chile. *J Nutr.* 2003;133:3166–3169.
14. Hirsch S, de la Maza P, Barrera G, et al. Chilean flour folic acid fortification program reduces serum homocysteine levels and masks vitamin B12 deficiency in elderly people. *J Nutr.* 2002;132: 289–291.
15. Institute of Medicine, Food and Nutrition Board. Folate. *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline.* Washington, DC: National Academy Press; 1998:196–303.
16. COMA and Report. Committee on Medical aspects of Food and Nutrition Policy. *Folic Acid and the Prevention of Disease.* London: The Stationery Office; 2000.
17. Mills JL, Von KohornI, Conley MR, et al. Low vitamin B-12 concentrations in patients without anemia: the effect of folic acid fortification of grain. *Am J Clin Nutr.* 2003;7:1474–1477.
18. Olivares M, Hertrampf E, Capurro MT, Wegner D. Prevalence of anemia in elderly subjects living at home of micronutrient deficiencies and inflammation. *Eur J Clin Nutr.* 2000;54:834–839.
19. Daly LE, Kirke PN, Molloy A et al. Folate levels and neural tube defects. Implications for prevention. *JAMA.* 1995;274:1698–702.
20. Daly S, Mills JL, Molloy AM, et al. Minimum effective dose of folic acid for food fortification to prevent neural tube defects. *Lancet.* 1997;350:1666–1669.