Wheat Flour Fortification With Folic Acid: Changes in Neural Tube Defects Rates in Chile

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In January 2000, Chilean Ministry of Health mandated the addition of folic acid (FA) to wheat flour in order to reduce the risk of neural tube defects (NTDs). This policy resulted in significant increases in serum and red cell folate in women of fertile age 1 year after fortification. To evaluate the effect of wheat flour fortification on the prevalence of NTDs in Chile we designed a prospective hospital-based surveillance program to monitor the frequency of NTDs in all births (live and stillbirths) with birth weight ≥500 g at the nine public maternity hospitals of Santiago, Chile from 1999 to 2009. During the pre-fortification period (1999–2000) the NTD rate was 17.1/10,000 births in a total of 120,566 newborns. During the post-fortification period (2001–2009) the NTD rate decreased to 8.6/10,000 births in a total of 489,915 newborns, which translates into a rate reduction of 50% (RR: 0.5; 95% CI: 0.42–0.59) for all NTDs. The rate reduction by type of NTD studied was: 50% in anencephaly (RR: 0.5; 95% CI: 0.38–0.67), 42% in cephalocele (RR: 0.58; 95% CI: 0.37–0.89), and 52% in spina bifida (RR: 0.48; 95% CI: 0.38–0.6). Rates showed significant reduction both in stillbirths and live births: 510.3 to 183.6/10,000 (RR = 0.36; 95% CI: 0.25–0.53) and 13.3 to 7.5/10,000 (RR = 0.56; 95% CI: 0.47–0.68), respectively.

In Chile, fortification of wheat flour with FA has proven to be an effective strategy for the primary prevention of NTDs.

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Key words: neural tube defects; folic acid; anencephaly; cephalocele

INTRODUCTION

Neural tube defects (NTDs) including anencephaly, cephalocele, and spina bifida, occurred in 17.1 of 10,000 births before folic acid (FA) fortification of wheat flour in Chile [Nazer et al., 2001]. NTDs are the second most frequent isolated birth defect after congenital heart defects and represent the first congenital malformation preventable through public health nutrition measures. The risk of having a fetus affected with an NTD can be reduced in 50–70% by maternal periconceptional intake of FA [MRC, 1991; Czeizel and Dudas, 1992]. Recommendations from North America [Centers for Disease Control and Prevention (CDC) 1992], Europe [Scientific Committee on Human Nutrition, 1993], and FAO/WHO (FAO/WHO expert consultation, 2002) are in agreement that all women in fertile age should consume 400 μg of FA daily to reduce their risk of having a pregnancy affected by NTDs.

FA supplementation and food fortification are being used to increase FA intake by women in fertile age. Prevention policies focused on the use of dietary supplements have not been effective in obtaining a sustained prevention of NTDs [Raats et al., 1998; De Walle et al., 1999; Botto et al., 2005], whereas fortification of cereals with FA has shown significant increase of blood folate levels and also significant reductions in NTDs frequencies in USA, Canada, South Africa, Costa Rica, Brazil, and Argentina [Honein et al., 2001; CDC, 2002; Persad et al., 2002; Williams et al., 2002; Chen and Rivera, 2004; De Wals et al., 2007; Sayed et al., 2008; López-Camelo et al., 2010], validating the decision to choose a universal intervention. In Chile where most pregnancies are unplanned, the reasonable approach to obtain adequate periconceptional levels of FA seemed to be through food fortification.

In January 2000, the Chilean Ministry of Health mandated the addition of 2.2 mg of FA/kg of wheat flour at the national level in
order to reduce the risk of NTD. Based on the average intake of bread in women of reproductive age, this amount delivered an estimated additional supply of FA close to international recommendations, and resulted in significant increases in serum folate and red cell folate of 3.8 and 2.4-fold, respectively, in women of fertile age 1 year after fortification [Hertrampf et al., 2003]. According to the Latin-American Collaborative Study of Congenital Malformations (ECLAMC) and to previous reports done by us, the rate of NTDs in Chile decreased 2 years after the FA fortification program was implemented [López-Camejo et al., 2005, 2010; Hertrampf and Cortés, 2004, 2008]. The effect of this intervention on the incidence of NTDs in Santiago of Chile was prospectively evaluated from a registry specially designed for this study. Final results are presented in this article.

**METHODOLOGY**

**Study Population**

Using a hospital-based design, we studied the frequency of NTDs in live births and stillbirths with weights ≥500 g at the nine public maternity hospitals of Santiago from 1999 to 2009. Theses hospitals are part of the National Health System, which takes care of people of low and middle socioeconomic level.

Santiago, Chile's capital city has 6,880,000 inhabitants, who represent 40.3% of the country's population. The average number of births occurring in the nine hospitals included in our study, 55,000 per year, corresponds to 25% of the annual births in the country.

Since the Chilean health system does not have a National Birth Defect Registry and birth certificates do not include information about congenital anomalies, data on cases of NTDs (anencephaly, spina bifida, and cephalocele) were obtained from a specially designed registry for this study.

Chile has a well-recognized public health system, with important outcomes such as: A low infant mortality rate of 7.9 per 1,000 births (year 2008), where, following premature births, the second leading cause of death among children less than 1-year-old are congenital anomalies. Most, 98.9%, of births occur with professional assistance or in an institutional setting (80% under the national health public system) and 99.8% of national births are included in a neonatal screening program for phenylketonuria and congenital hypothyroidism [MINSAL, 2003, 2009, 2011]. Consequently, nearly all newborns are professionally examined, allowing for an easy ascertainment of NTDs. Moreover, in Chile newborns with spina bifida are discharged after been evaluated and treated. Other important factors that contribute to a high rate of ascertainment of NTDs in this study is that all cases of stillbirths must be registered and audited to determine the cause of death, and termination of pregnancies is prohibited by law under any circumstance.

In each of the nine hospitals a contact professional (medical doctor or a midwife) was specially trained by the research team to review the daily delivery records, to register and examine all births affected by NTDs. This primary information was reviewed by the research team using the following sources: Registry of newborns with congenital anomalies, registry of hospitalized newborns, hospital discharge reports, registry of stillbirths, fetal death audits, registry of deaths of children under 1 year, autopsy protocols, and clinical records.

Throughout the follow-up period, data collected through the described sources were reviewed monthly in each hospital by a clinical geneticist from the research team. Validation of the collected data was performed by the rest of the research team through the above described sources every 3 months.

The NTDs monitored include: Anencephaly, cephalocele and open spina bifida, presented as an isolated defect or associated with other congenital defects, unrelated to NTDs. To avoid double-registry of cases with multiple NTDs, when a newborn had two or more NTDs, the upper one was considered. NTDs produced by amniotic bands and occult spina bifida were not included.

Cases of NTDs were categorized as simple if only one NTD was present or complex when more than one NTD was detected.

The years of follow-up were grouped as pre-fortification period from 1999 to 2000 and as post-fortification period from 2001 to 2009. These periods were established considering that FA fortification was mandatory by national law in January 2000, but that hard evidence of fortification of the wheat flour consumed in Santiago was not obtained until April 2000, when random samples of bread where analyzed [Hertrampf et al., 2003].

This study was conducted with the approval of the Investigational Review Board of the Institute of Nutrition and Food Technology (INTA), University of Chile.

**Statistical Analysis**

The prevalence of each type of NTD was calculated per 10,000 births, using the number of all births with NTDs (live births and stillbirths) as the numerator, and the total number of births (all live and stillbirths) in the included public hospitals as the denominator.

To make our approach comparable with previous studies, relative risks for each type of NTD was calculated for the prevalence in the post-fortification period (2001–2009) and in the pre-fortification period (1999–2000). Ninety-five percent confidence intervals (95% CI) around these ratios were calculated using EPI-INFO 6.

Chi-square test was used to analyze differences between proportions. Joint point regression [Kim et al., 2000] was made to evaluate the trend for the total number of NTD diagnosed per year (1999–2009) in both stillbirths and live births using Jointpoint Regression Program version 3.4.2 [NCI, 2009]. All P-values were two-tailed, and a value of <0.05 was considered to be statistically significant.

**RESULTS**

During the period under surveillance (1999–2009), there were 625 NTDs cases (219 with anencephaly, 309 with spina bifida, and 97 with cephalocele) reported among 610,481 total deliveries (120,566 births in the pre-fortification period and 486,779 in the post-fortification period).

The total NTD rate decreased from 17.1/10,000 births in the pre-fortification period (1999–2000) to 8.6/10,000 births in the post-fortification period (2001–2009) (RR = 0.5; 95% CI = 0.42–0.59). Table I compares the pre and post-fortification rates of specific
NTDs (anencephaly, cephalocele, and spina bifida). Spina bifida and anencephaly showed similar significant decrease, 52% (RR = 0.48; 95% CI = 0.38–0.67; P-value < 0.001) for spina bifida and 50% (RR = 0.50; 95% CI = 0.38–0.67; P-value < 0.001) for anencephaly. The decrease for cephalocele was 42% (RR = 0.58, 95% CI = 0.37–0.89, P-value < 0.001).

When considering the fetal condition at birth, a reduction of the NTDs rate was observed in both live births and stillbirths. In live births the reduction of the NTDs rate was from 13.3 to 7.5/10,000 live births (RR = 0.56, 95% CI = 0.47–0.68) and in stillbirths NTDs rate dropped from 510.3 to 183.6/10,000 (RR = 0.36, 95% CI = 0.25–0.53). Among live births the most common defect was spina bifida and its rate dropped from 8.6 to 3.4/10,000 live births (RR = 0.46, 95% CI = 0.36–0.58; P < 0.0001). Cephalocele dropped from 2.3 to 1.3/10,000 live births (RR = 0.56, 95% CI = 0.36–0.89; P = 0.012) and there was no variation in anencephaly rates in this group. The most common defect among stillbirths was anencephaly and its prevalence dropped from 466.9 to 124.6/10,000 stillbirths (RR = 0.27, 95% CI = 0.17–0.41; P < 0.001); however, there was no statistical variations in the rates of cephalocele or spina bifida in this group (Fig. 1).

Both simple and complex NTDs prevalence decreased. The most significant reduction was seen in complex NTDs. Simple NTDs dropped from 14.9 to 8/10,000 births and complex NTDs decreased from 2.2 to 0.6/10,000 births.

The Joint Point Regression test showed that annual NTD rates dropped since 2002 and then increased since 2007. The events after 2007 affect specially the group of live births (Fig. 2). This effect was significant just for two of the nine hospitals and only for spina bifida (P = 0.018 and 0.048). In five of the hospitals the frequency was stable and in the other two the number of spina bifida continued dropping significantly (P = 0.003 and 0.016).

**DISCUSSION**

This study demonstrate a significant reduction in the prevalence of NTDs in a population of women of middle and low socioeconomic status users of the hospitals from the Chilean National Health System in which 70% of national births occur. This reduction was temporally associated with wheat flour fortification with FA and was observed within a very short period of time after fortification started as suggested in preliminary reports [Hertrampf and Cortés, 2004, 2008].

This is the second registry that shows a decrease in the birth prevalence of NTDs subsequent to FA wheat flour fortification in Chile. Data from the ECLAMC [López-Camelo et al., 2005], including 25 Chilean hospitals showed 585 NTD cases (only considering anencephaly and spina bifida) among 352,127 births (16.6/10,000) between 1982 and 2000, a period with no food fortification. In the period 2001–2002 after the fortification program started, the birth prevalence rate of NTD’s decreased to 90 NTDs cases in 113,268 births (7.9/1,000). In that report, the post-fortification population studied was relatively small and included only a few years of follow-up [López-Camelo et al., 2005]. In 2010...
the ECLAMC [López-Camelo et al., 2010] reported its data until 2007 including cephaloceles and a post-fortification population of 243,624 births, showing a significant reduction of birth prevalence of all monitored NTDs. Prevalence rate ratios in post-fortification period were 0.52 for anencephaly (95% CI: 0.36–0.83, \( P < 0.004 \)), 0.43 for spina bifida (95% CI: 0.31–0.6, \( P < 0.001 \)) and 0.47 for cephalocele (95% CI: 0.27–0.83, \( P = 0.009 \)) [López-Camelo et al., 2010]. The ECLAMC’s results confirmed a protective effect of FA fortification for anencephaly, spina bifida, and also for cephalocele. In the present study, including 610,481 births in Santiago de Chile, with 9 years of surveillance post-fortification and considering data from anencephaly, spina bifida, and cephalocele, the results are concordant with the ECLAMC’s reports.

Our results showed that 50% of the potentially new cases of NTDs were prevented (preventable fraction) with similar reduction for spina bifida (52%) and anencephaly (50%), and less effect in

**FIG. 2.** Joint point regression analysis of annual NTDs rates [a] total births, [b] stillbirths, and [c] live births.
rate of 17.1 per 1,000 births. As cephalocele is a type of NTD that is more frequently a feature of a “genetic syndrome”, monogenic, or chromosomal, FA will have a lesser effect [Stevenson et al., 2000; Siffel et al., 2003].

Also important reductions in NTDs rates have been reported in the United States [Mathews et al., 2002; CDC, 2004; Williams et al., 2005], Canada [Persad et al., 2002; De Wals et al., 2007], Costa Rica [Chen and Rivera, 2004], and South Africa [Sayed et al., 2008], following fortification of flour with FA. In Canada, there was a 38% reduction in anencephaly, 52% in spina bifida, and 31% in cephalocele [De Wals et al., 2007]. In the United States, data from birth defects monitoring programs with prenatal ascertainment of cases showed a reduction of approximately 17% for anencephaly and 36% for spina bifida [Siffel et al., 2003]. No data for cephaloceles were presented in that study. In South Africa, there was a 10.9% reduction in anencephaly and 41.6% in spina bifida, but not in cephaloceles [Sayed et al., 2008].

In Canada, USA, and South Africa, the rate reduction in spina bifida in association with fortification has been consistently greater than the reduction in anencephaly [Mathews et al., 2002; Williams et al., 2005; De Wals et al., 2007; Sayed et al., 2008]. A similar reduction in spina bifida and anencephaly has been reported in Chile by ECLAMC and by this study. Similar reduction in cephalocele rates also are showed in live births in Canada after FA food fortification [De Wals et al., 2007]. Our findings also show a reduction in both simple and complex NTDs, but the effect is neither evident in live births nor in stillbirths when these groups are analyzed separately.

The strengths of this study include its prospective design, the inclusion of stillbirths with birth weight of $\geq$500 g, and the current Chilean legislation prohibiting abortion, which increases the possibility of under-registration due to interruptions of pregnancy. The pre-fortification NTDs rate found in this study (17.1/1,000 births) was the same obtained by ECLAMC, this fact further strengthens the reliability of our surveillance system. Although illegal abortion does exist in Chile, data about its frequency are not available; however, indirect evidence on maternal mortality due to abortion and puerperal sepsis rates support the idea that the number of illegal abortions has not changed in the last decade. Maternal mortality in Chile showed a reduction of 60.3% between 1990 and 2000 and there was also a significant downward trend in maternal mortality due to abortion between 1985–1990 was constant, between 30 and 40 deaths per year, decreasing to 10 deaths per year by 1994 and no deaths reported between 1997 and 2000. [Donoso, 2004]; all this epidemiological data suggest that the number of induced abortions related to NTDs is negligible if and if they exist, their frequency should not have changed over time. Although, as interruption of pregnancy is illegal, we do not have data about changes in the procedure such as improvement of sterile techniques that could also explain the decrease in maternal mortality due to abortion.

The temporal association of the decrease in NTDs rates with the implementation of FA fortification, the estimated increase in the mean consumption of FA from bread (427 $\mu$g/day), and the important increase of folate blood levels in Chilean women of fertile age 1 year after fortification are strong arguments for a causal association between the decrease of NTD and FA fortification of wheat flour [Hertrampf et al., 2003]. Also in support of a causal relation, is the fact that, according to ECLAMC data, fortification was implemented when secular trends of NTDs were increasing [Nazer et al., 2001], contrasting with the historically decreasing rates observed in industrialized countries.

Results of an economic analysis of the Chilean program of wheat flour fortification with FA performed by our group [Llanos et al., 2007] showed that its implementation, in addition to being effective, is cost-saving. The annual total cost of the fortification program, covering the entire country, corresponds to the cost of the rehabilitation program of two individuals with NTD from birth until adulthood. The cost-benefit ratio in averting NTDs was 10 to 1 in Chile, in South Africa 30 to 1 [Sayed et al., 2008], and in the USA 40 to 1 [Grosse et al., 2005].

We suggest that in order to complement Chilean fortification programs, health professionals should offer FA supplementation to women with high recurrence risk of NTDs (parent affected by a NTD, previous child with NTD) or with high risk for NTDs (women taking antiepileptic drugs, women with diabetes mellitus). This study also reports a tendency to revert the decrease in NTD rates in live births, during 2008 and 2009. This phenomenon was observed clearly just in two of the nine hospitals included in the study and only for spina bifida. As the surveillance protocol has been the same throughout the period of observation, the increase in NTD frequency detected in two of the surveyed hospitals is most probably the result of other factors. One could be the increase of migrant population in the areas of the city covered by these two hospitals from countries, such as Peru, Bolivia, and Ecuador, whose traditional diets include a major proportion of corn flour, which is not fortified, than wheat flour. Other possibilities to be considered are the reported increased rates of diabetes among Chilean women, from 3.8% in 2003 to 10.4% in 2009, and of obesity, from 27% in 2003 to 30.7% in 2009, and the campaign “against eating bread” in order to reduce the levels of overweight and obesity. [MINSAL, 2003; MINSAL, 2009, Martínez, 2003].

Recent legislation mandated the reduction of FA level from 2.2 mg/kg to 1.8 mg/kg of wheat flour, which will be implemented in January 2012. This modification was based on high levels of serum folates found on elderly population [Sánchez et al., 2010] showing that groups of population are receiving unnecessarily high intakes of FA. It is important to be cautious when the whole population is exposed to an increase of FA intake, especially when some issues like the possible dual effect of this nutrient (high and low intakes) in some cancers as not has not been clarified. We think that it is necessary to continue with the observation in Chilean population.

In conclusion, Chilean public health conditions create a special situation to facilitate the evaluation of the impact of a food fortification program. An analysis of the economic impact of the fortification program showed that the reduction in the frequency of NTDs has been highly cost-effective. Our experience could be used by other countries choosing the ideal food staples and levels of fortification for their populations, closely monitoring the fortification process and the effectiveness of the program, and carefully surveying for possible adverse effects.
REFERENCES


