Effects of Folic Acid Fortification on Spina Bifida Prevalence in Brazil

Iêda Maria Orioli,[1,2]* Ricardo Lima do Nascimento,[1,2] Jorge Santiago López-Camelo,[2,3] and Eduardo Enrique Castilla[2,3,4]

[1]Estudio Colaborativo Latino Americano de Malformaciones Congénitas, at Departamento de Genética, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
[2]Instituto Nacional de Ciência e Tecnologia Genética Médica Populacional (INAGEMP), Rio de Janeiro, Brazil
[3]Estudio Colaborativo Latino Americano de Malformaciones Congénitas at Centro de Educación Médica e Investigaciones Clínicas, Buenos Aires, Argentina
[4]Estudio Colaborativo Latino Americano de Malformaciones Congénitas at Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

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BACKGROUND: To assess spina bifida birth prevalence changes after folic acid fortification of wheat and maize flours began in Brazil in June 2004. **METHODS:** Cross-sectional study of Brazilian live births in 2004 and 2006. Spina bifida birth prevalence from the Live Births Information System (SINASC: Sistema de Informações sobre Nascidos Vivos) in a prefortified period was compared to a period fortified with folic acid in each state. Observed prevalence rates in 2004 were used to calculate the expected prevalence rates in 2006 under the null hypothesis that both were similar. The observed/expected (O/E) ratios were tested by two-tailed Z-test. To minimize ascertainment differences among states, the O/E ratio of each one of the 27 Brazilian states was adjusted for the number of births with the Mantel-Haenszel statistic. **RESULTS:** The reduction in spina bifida birth prevalence in 2006 was 39% (O/E = 0.61; 95% confidence interval [CI], 0.55-0.67), and 40% (O/E = 0.60; 95% CI, 0.53–0.68), after adjusting for state birth number. This reduction was significant ($p < 0.0001$), and heterogeneous among states ($\chi^2 = 72.96$; $p < 0.0001$). **CONCLUSIONS:** Using SINASC data, there was a significant reduction in spina bifida birth prevalence in Brazil, probably related to the folic acid food fortification program. *Birth Defects Research (Part A) 91:831–835, 2011.* © 2011 Wiley-Liss, Inc.

Key words: folic acid; neural tube defect; spina bifida; food fortification; congenital defect; SINASC

INTRODUCTION

In Brazil, since 1999, the Live-Birth Form (in Portuguese ''declaração de nascido vivo'': DN) has included information on congenital anomalies. DN data are collected and codified by municipal or state health secretaries and, when considered complete, the data are sent to the Brazilian Health Ministry. The data are then disseminated using the Internet by the SUS Informatics Department, a section of the Health Ministry Executive Secretariat. The DN form presents alternative answers to the question about the occurrence of congenital anomalies in the newborn: *Yes*, *No*, and *Not Further Specified* (Ministério da Saúde, 2009). If the answer is *Yes*, each newborn anomaly must be completely described and codified subsequently using the ICD-10. Validated and properly managed, this instrument can be used to monitor congenital anomalies at the national level (Luquetti and Koifman, 2009), covering more than 3 million births annually.

Spina bifida (SB) is the most prevalent neural tube defect (NTD) presenting in South America together with anencephaly and cephalocele, a birth prevalence of approximately 1.5:1000 births (Castilla and Orioli, 1985; Nazer et al, 2001). The worldwide divergence in the NTD birth prevalence is the result of several underlying factors, such as geographic region, ethnicity, and socioeconomic factors (Elwood and Elwood, 1980), but the main reasons for the current variation are NTD prenatal detection and the different laws concerning voluntary interruption of pregnancy among countries (EUROCAT Working Group, 1991). NTDs are etiologically heterogeneous and isolated SB (i.e., without unrelated defects in

*Correspondence to: Iêda Maria Orioli, Departamento de Genética, Universidade Federal do Rio de Janeiro, Caixa Postal 68.011, 21941-000, Rio de Janeiro, Brazil. E-mail: orioli@centroin.com.br
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the same child) although more homogeneous (López-Camelo et al, 2010), has a complex etiology involving the interaction of genetic and environmental factors (Elwood and Elwood, 1980).

Gestational folic acid deficiency is considered an NTD risk factor, and there are several papers (Freire et al, 2000; Honein et al, 2001; Gucciardi et al, 2002; Persad et al, 2002; Williams et al, 2002; Hertrampf et al, 2003; López-Camelo et al, 2005; De Wals et al, 2007; Sayed et al, 2008; Berry et al, 2010) reporting an increase in blood folate and a reduction in NTD birth prevalence after folic acid food fortification in several populations. However, there have been few studies in Brazil regarding the results of folic acid fortification of flours after the Health Ministry regulatory resolution in 2003 (Agência Nacional de Vigilância Sanitária, 2002; Santos and Pereira, 2007). In addition, there is scarce information about Brazilian regional differences in the effective folic acid dose ingested by the Brazilian population (Ferreira and Giugliani, 2008), which is important because there are regional differences in dietary habits.

Our aim was to verify changes in the SB birth prevalence using the SINASC: Sistema de Informações sobre Nascidos Vivos (Live Birth Information System) data available on the Internet after the fortification of wheat and corn flours with folic acid began in Brazil, in June 2004 (Agência Nacional de Vigilância Sanitária, 2002).

MATERIALS AND METHODS

The fortification of Brazilian flour with folic acid began in June 2004, and fortified births must have occurred from June 2005 onward. The 12-month period from the beginning of maternal fortification to the birth of the first children included a 3-month period for the implementation of flour fortification, and an additional 9 months of gestation. In addition, the second half of 2005 was not used as a fortified period in this study since the Internet data could not be split by semesters. Consequently, we analyzed data from the years 2004 and 2006. We calculated the SB birth prevalence in each one of the 5627 Brazilian municipalities, distributed among 27 states (i.e., 26 states and one Federal District). The number of Brazilian municipalities changes annually, and we used the number existing in SINASC data for the year 2004. SB (code Q.05 in the *International Classification of Diseases*, edition 10) is one of the diagnoses available on the Internet at http://www2.datasus.gov.br/DATASUS/index.php?area=0205&VObj=http://tabnet.datasus.gov.br/cgi/deftohtm.exe?sinasc/cnv/nv. The data were retrieved on March 18th, 2009, and comprised 634 SB cases among 2,747,277 live births in 2004 and 393 SB cases among 2,798,949 live births in 2006. The births for which congenital anomalies were not specified further were excluded. Municipality was the unit of analysis, and SB birth prevalence per 10,000 live births was calculated for both periods.

To evaluate the effect of folic acid fortification on the SB birth prevalence, the 2006 rates (in the fortified period) were compared to the 2004 rates (prefortified or reference period). According to the SB prevalence observed in 2004 in each state, the expected prevalence for 2006 was calculated, under the null hypothesis of no differences between 2004 and 2006 rates. The observed/expected (O/E) ratios, with 95% confidence intervals and the two-tailed Z test ($O-E/E^{1/2}$) after the Poisson distribution, were calculated to identify states with an increased or decreased SB birth prevalence at the significance level of 0.001, chosen after Bonferroni's adjustment.

To minimize variation in SB ascertainment in each state, the O/E values were adjusted for the year 2006. First, we estimated the 2006 O/E ratios using the 2004 birth prevalence as the expected value in each state. Second, these O/E ratios were weighted by the number of births in the state, and the adjusted O/E ratios were obtained with the Mantel-Haenszel test (Armitage and Berry, 1994). The heterogeneity of O/E ratios among states was determined with the chi-square test with 25 degrees of freedom.

To determine the effect of the small sample size on the variation in SB ascertainment, the 5627 municipalities were grouped according to their number of births in five intervals: (1) without births or not further specified number of births; (2) 1 to 999 births; (3) 1000 to 4999 births; (4) 5000 to 9999 births; and (5) more than 10,000 births. The SB birth prevalence and the O/E ratios for each year (2004 and 2006) were calculated within each stratum. The regression coefficients between birth prevalence or O/E ratios, or both, and birth number were calculated using Mann-Kendall nonparametric test. Furthermore, these O/E ratios were weighted by the number of births in each stratum and the adjusted O/E ratios obtained with the Mantel-Haenszel test (Armitage and Berry, 1994). The heterogeneity of O/E ratios among strata was determined with the chi-square test with three degrees of freedom.

To verify whether any modification in SB prevalence was not caused by an underlying trend in reducing NTD rates from before folic acid supplementation, we analyzed the total SB prevalence in Brazil for 2000 to 2004 before beginning fortification with the Mann-Kendall nonparametric test.

RESULTS

The reduction in SB birth prevalence in 2006 was 39% (95% CI, 33 to 45%; O/E = 0.61; 95% confidence interval [CI], 0.55−0.67), as shown in Table 1. When adjusted by the number of births in each state, this reduction was 40% (95% CI, 32−47%), and the O/E was 0.60 (95% CI, 0.53−0.68; $p < 0.001$). This reduction was statistically significant ($p < 0.001$) and heterogeneous among states ($\chi^2 = 152.4$; $p < 0.001$). Considered individually, only one state (Bahia), presented an increased SB birth prevalence, whereas five states had statistically significant decreases for SB occurrences—Minas Gerais, Pernambuco, Paraná, São Paulo, and Alagoas—besides the country as a whole. The states of Acre, Amapá, and Roraima had no SB cases in the year 2004, nor did the Distrito Federal in the year 2006.

Table 2 shows the SB birth prevalence in 2004 and 2006 and the O/E ratios according to the municipal number of births, in five categories. There was a significant regression coefficient (b) between the SB birth prevalence and the number of births for both studied periods. Municipalities with more live births had a higher SB prevalence than did municipalities with fewer live births (2004: $b = 0.39$; $p < 0.001$; and 2006: $b = 0.33$; $p < 0.001$). Independent of live birth numbers, the SB birth prevalence was lower in 2006 than in 2004 in all categories, showing a total reduction in SB birth prevalence of 39%

Table 1
Effect of Folic Acid Fortification on the Spina Bifida Birth Prevalence in 27 Brazilian States

	2004			2006								
States[a]	No. of cases	No. of births	Prevalence per 10,000	No. of cases	No. of births	Prevalence per 10,000	E	O/E	95% CI		Z	*p*
AC	0	16,430	0.00	1	16,382	0.61	0.00	—	—	—	—	
AL	8	55,181	1.45	1	54,151	0.18	7.85	0.13	0.00	0.71	−2.45	<0.05
AM	9	67,178	1.34	9	72,284	1.25	9.68	0.93	0.42	1.76	−0.22	
AP	0	14,170	0.00	1	15,191	0.66	0.00	—	—	—	—	
BA	16	207,053	0.77	28	196,201	1.43	15.16	1.85	1.22	2.66	3.30	<0.01
CE	14	127,614	1.10	10	120,186	0.83	13.19	0.76	0.36	1.39	−0.88	
DF	2	25,483	0.78	0	38,508	0.00	3.02	0.00	0.00	0.01	−1.74	
ES	3	51,071	0.59	2	51,086	0.39	3.00	0.67	0.08	2.41	−0.58	
GO	15	77,849	1.93	10	75,514	1.32	14.55	0.69	0.33	1.26	−1.19	
MA	9	114,161	0.79	6	120,727	0.50	9.52	0.63	0.23	1.37	−1.14	
MG	52	252,414	2.06	29	250,457	1.16	51.60	0.56	0.38	0.81	−3.15	<0.001
MS	3	40,441	0.74	5	39,061	1.28	2.90	1.73	0.56	4.02	1.24	
MT	11	50,993	2.16	9	49,373	1.82	10.65	0.85	0.38	1.60	−0.51	
PA	16	141,325	1.13	17	148,679	1.14	16.83	1.01	0.59	1.62	0.04	
PB	9	57,296	1.57	6	58,504	1.03	9.19	0.65	0.24	1.42	−1.05	
PE	49	148,107	3.31	27	145,908	1.85	48.27	0.56	0.37	0.81	−3.06	<0.001
PI	2	56,550	0.35	3	57,231	0.52	2.02	1.48	0.31	4.38	0.69	
PR	30	159,605	1.88	15	153,212	0.98	28.80	0.52	0.29	0.86	−2.57	<0.05
RJ	48	207,061	2.32	39	200,253	1.95	46.42	0.84	0.60	1.15	−1.09	
RN	18	48,449	3.72	12	46,452	2.58	17.26	0.70	0.36	1.21	−1.27	
RO	1	28,467	0.35	3	24,698	1.21	0.87	3.46	0.69	9.74	2.29	
RR	0	9,643	0.00	0	9,539	0.00	0.00	—	—	—	—	
RS	32	152,146	2.10	39	139,776	2.79	29.40	1.33	0.94	1.81	1.77	
SC	15	84,867	1.77	12	83,803	1.43	14.81	0.81	0.42	1.42	−0.73	
SE	11	33,139	3.32	11	37,501	2.93	12.45	0.88	0.44	1.59	−0.41	
SP	259	494,933	5.23	94	568,223	1.65	297.35	0.32	0.26	0.39	−11.79	<0.001
TO	2	25,651	0.78	4	26,049	1.54	2.03	1.97	0.55	5.12	1.38	
Total[b]	634	2,747,277	2.31	393	2,798,949	1.40	645.92	0.61[c]	0.55	0.67	−9.95	<0.001

[a]States are Brazilian Federal Units: AC, Acre; AL, Alagoas; AM, Amazonas; AP, Amapá; BA, Bahia; CE, Ceará; DF, Distrito Federal; ES, Espírito Santo; GO, Goiás; MA, Maranhão; MG, Minas Gerais; MS, Mato Grosso do Sul; MT, Mato Grosso; PA, Pará; PB, Paraíba; PE, Pernambuco; PI, Piauí; PR, Paraná; RJ, Rio de Janeiro; RN, Rio Grande do Norte; RO, Rondônia; RR, Roraima; RS, Rio Grande do Sul; SC, Santa Catarina; SE, Sergipe; SP, São Paulo; TO, Tocantins.

[b]Heterogeneity $\chi^2 = 72.96$; $p < 0.0001$; 25 degrees of freedom.

[c]Mantel-Haenszel O/E = 0.60; 95% CI, 0.53−0.68. E, expected number; O/E, observed/expected ratio; CI, confidence intervals.

(O/E = 0.61). No significant regression coefficient was observed between the O/E ratios and the municipality live birth numbers ($\chi^2 = 2.31$; $p < 0.129$; three degrees of freedom). The adjusted reduction in SB prevalence was 41% (95% CI, 33−48%), and the O/E was 0.59 (95% CI, 0.52−0.67; $p < 0.001$). This reduction was statistically significant ($p < 0.0001$) and homogeneous among the four strata ($\chi^2 = 7.39$; $p = 0.06$; three degrees of freedom).

The SB prevalence per 10,000 in Brazil was 1.40 (403/2,873,047) in the year 2000, 1.82 (505/2,768,412) in 2001, 1.81 (502/2,770,679) in 2002, 2.05 (566/2,755,440) in 2003, and 2.31 (634/2,747,277) in 2004. There was a significant increasing trend of SB prevalence between the years 2000 and 2004 ($b = 0.22$; $z = 2.43$; $p < 0.05$).

DISCUSSION

Our results show a temporal decline in SB prevalence in relation to folic acid fortification, with observed protection of between 32 and 47% using data adjusted for the states' live birth numbers. When the municipalities were grouped in strata by number of births and the calculated reduction was adjusted by births in the four specified strata, the observed protection of between 33 and 48% remained similar to the previous calculations.

The observed increasing trend in SB prevalence in the first 5 years before beginning fortification has already been described using hospital births examined by Estudio Colaborativo Latino Americano de Malformaciones Congénitas (ECLAMC) between January 2003 and June 2005 (López-Camelo et al., 2010). The SB prevalence in this hospital-based series (López-Camelo et al., 2010), which included live and still births, was 6.3-fold higher than the SB prevalence registered by SINASC in live births. As a result, some improvement in the SB registration by the SINASC could be contributing to the ascending time trend.

Although the SB prevalence reduction was not statistically significant in all states, only one of the 27 states showed an increasing trend. Three states did not report cases. The country as a whole presented a significant decrease against an expected increasing trend as mentioned before.

There are several difficulties in comparing these Brazilian data for a reduction in SB birth prevalence with the values published in the literature for other countries. For example, there are differences in the doses of folic acid used in the fortification, as well as in the date when forti-

Table 2
Effect of Folic Acid Fortification on the Spina Bifida Birth Prevalence, According to Brazilian Municipalities Stratified by Birth Number

Total births	2004						2006							
	No. of municipalities	No. of SB cases	No. of specified births	No. of NS	NS births %	SB prevalence per 10,000	No. of municipalities	No. of SB cases	No. of specified births	No. of NS	NS births %	SB prevalence per 10,000	E	O/E
0[a]	1156	0	0	0	—	—	1370	0	0	0	—	—	—	—
1–999	3932	63	735,820	48,390	6.17	0.86	3725	54	705,460	23,657	5.72	0.77	60.40	0.89
1000–4999	458	166	851,227	60,411	6.63	1.95	447	97	847,590	40,011	3.34	1.14	165.29	0.59
5000–9999	49	87	325,412	26,497	7.53	2.67	50	38	324,753	7,256	1.39	1.17	86.82	0.44
≥10000	32	318	834,818	143,973	14.71	3.81	35	204	921,146	75,055	6.92	2.21	350.88	0.58
Total[b]	5627	634	2,747,277	279,271	9.23	2.31	5627	393	2798,949	145,979	4.96	1.40	645.92	0.61[c]

[a]Only municipalities with no births.
[b]Heterogeneity $\chi^2 = 7.39$; $p = 0.06$; three degrees of freedom.
[c]Mantel-Haenszel O/E = 0.59, 95% CI, 0.52–0.67. SB, spina bifida; NS, births not further specified for congenital defects; E, expected; O/E, observed/expected ratio.

fication began, in the diligence in following the law or the regulation about fortification, and in the degree of congenital malformation ascertainment. In addition, termination of pregnancy due to spina bifida is illegal in Brazil.

This cross-sectional study examined SB in the Brazilian live birth population in two periods differing by the absence and presence of folic acid food fortification, which were then subjected to the ecologic fallacy—that is, we grouped the births under the assumption of whether they were fortified with folic acid without knowing for sure about each mother's diet during the pregnancy. The data suggest a temporal association between folic acid flour fortification and the reduction in SB birth prevalence in Brazil. Because of the continental size of the country, the numerous factors previously cited as difficulties when comparing Brazilian statistics for reduction rates with those of other countries could exist internally in Brazil, contributing to differences among states that are not easily corrected. Although the SB birth prevalences were adjusted for the number of births, other factors such as ethnicity, which may have been related to SB prevalence, were not considered (Elwood and Elwood, 1980).

Reviews of the literature (Eichholzer et al, 2006; Santos and Pereira, 2007) concerning the obligatory fortification of food with folic acid in the United States, Canada, Costa Rica, Chile, and Australia showed a reduction in NTD prevalence of between 19 and 78%. Another review (Botto et al, 2006) examined data from population-based birth defects registries in Europe, North America, and Australia and concluded that folic acid food fortification appeared to be effective in NTD prevention, but its effect on other congenital defects was not clear. However, a systematic review of the efficiency of folic acid fortification in NTD reduction found controversial and inconclusive results for other parts of the world (Leoncini and Mastroiacovo, 2009).

In South America, Chile and Argentina have adopted the same folic acid concentration in wheat flour (2.2 mg/kg), whereas Brazil uses only 1.5 mg/kg and also has fortified corn flour. There is variation in the consumption of wheat flour in each of the three countries, with the average ingested daily dose of folic acid being calculated as 499 μg in Chile (Hertrampf et al, 2003), 486 μg in Argentina (Calvo and Biglieri, 2008; Zabala et al, 2008), and 264 μg in Brazil (Ferreira and Giugliani, 2008). The year in which fortification began also varied. Chile has been fortifying wheat flour with folic acid since January 2000, Argentina since November 2003, and Brazil since July 2004. The last published analysis based on information recorded by ECLAMC (Castilla and Orioli, 2004), comparing NTD birth prevalence in the three countries, in the prefortified and fortified periods, showed a reduction of anencephaly and of isolated SB (without unrelated defects in the same child) in the three countries and a reduction of cephalocele only in Chile (López-Camelo et al, 2010). López-Camelo et al (2010) also found a greater reduction in birth prevalence for high SB (cervical and thoracic) compared with lower level SB (lumbar and sacral), besides a greater reduction in SB than in anencephaly as demonstrated in Chile (López-Camelo et al, 2005), as well as in other parts of the world, including the United States (Williams et al, 2002), Canada (De Wals et al, 2007), and South Africa (Sayed et al, 2008).

We studied only SB for two reasons. First, SB is the most sensitive to folic acid fortification among NTDs (López-Camelo et al, 2010). Second, anencephaly has a high mortality rate resulting in approximately 50% of cases not being registered in the Brazilian DN. However, we were not able to subdivide SB in high and low types or discard the associated cases of SB in our analysis, because this kind of information is not available from the SUS Informatics Department via the Internet.

The previous finding of a reduction in SB birth prevalence in Brazil was 48% (24–65%; López-Camelo et al, 2010), similar to what was found in this study (40%; IC, 32–47%). It is worth mentioning that our previous work used information recorded by the ECLAMC after the examination of 1,107,514 live and still births occurring in 19 Brazilian hospitals, whereas we used the national total of live births for each year. Although the ECLAMC information has been considered the gold standard for birth defects ascertainment and registry (Luquetti and Koifman, 2009), the easy availability of the information all over the country partially compensates for the data deficiencies of SINASC.

Pacheco et al (2009), also using SINASC data, but including anencephaly and cephalocele in addition to spina bifida, compared the period of 2000 to 2004 with 2005 to 2006 in Recife, the capital of the state of Pernambuco in Brazil, and found no reduction in NTD birth prevalence. In this study, we compared only the year 2004 versus the year 2006 to avoid the greater subregistration or underascertainment expected to occur in the first few years after the congenital defect field had been included in the DN form. In our analysis, we found a statistically significant reduction of 44% in the SB birth prevalence rate for the entire state of Pernambuco; if we use the period of 2000 to 2004 and compare it with 2005 to 2006, the same period used by Pacheco et al (2009), we find a reduction in SB birth prevalence of 37%, which is not statistically significant. These analyses suggest that there was subregistration of SB in the early years of congenital anomalies registration by SINASC, resulting in an expected prevalence lower than the real one. In addition, the inclusion of the whole of 2005 in the fortified period, by Pacheco et al (2009), must have contributed to their negative results, because the fortification began in July 2004 in Brazil and probably did not affect births in the first 6 months of 2005.

CONCLUSION

Our positive results concerning the reduction in SB birth prevalence after folic acid fortification in Brazil confirmed previous results (López-Camelo et al, 2010) obtained with ECLAMC information from 19 Brazilian hospitals. To our knowledge, this study is the first to use all the national data (DATA-SUS, SINASC) concerning a congenital anomaly available through the Internet, to test the efficacy of a specific public health action: the fortification of wheat and corn flours with folic acid. We also confirmed the importance of the DN for monitoring congenital defects in this country.

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