RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Folic Acid Flour Fortification: Impact on the Frequencies of 52 Congenital Anomaly Types in Three South American Countries

Jorge S. López-Camelo,[1,2] Eduardo E. Castilla,[2,3] Iêda M. Orioli[4]* and INAGEMP (Instituto Nacional de Genética Médica Populacional) and ECLAMC (Estudio Colaborativo Latino Americano de Malformaciones Congénitas)

[1]IMBICE: Instituto Multidisciplinario de Biologia Celular, La Plata, Argentina

[2]CEMIC: Centro de Educación Médica e Investigación Clínica, Buenos Aires, Argentina

[3]Laboratório de Epidemiologia de Malformações Congênitas, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

[4]Laboratório de Malformações Congênitas, Departamento de Genética, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Received 13 September 2009; Accepted 29 March 2010

The aim of the present investigation was to search for a reduction in birth prevalence estimates of 52 selected types of congenital anomalies, associated with folic acid fortification programs in Chile, Argentina, and Brazil. The material included 3,347,559 total births in 77 hospitals of the three countries during the 1982–2007 period: 596,704 births (17 hospitals) in Chile, 1,643,341 (41 hospitals) in Argentina, and 1,107,514 (19 hospitals) in Brazil. We compared pre- and post-fortification rates within each hospital and the resulting Prevalence Rate Ratios (PRRs) were pooled by country. Statistically significant reductions in birth prevalence estimates after fortification were observed for neural tube defects (NTDs), septal heart defects, transverse limb deficiencies, and subluxation of the hip. However, only the reduction of NTDs appeared to be associated with folic acid fortification and not due to other factors, because of its consistency among the three countries, as well as with previously published reports, and its strong statistical significance. Among the NTDs, the maximum prevalence reduction was observed for isolated cephalic (cervical-thoracic) spina bifida, followed by caudal (lumbo-sacral) spina bifida, anencephaly, and cephalocele. This observation suggests etiologic and pathogenetic heterogeneity among different levels of spina bifida, as well as among different NTD subtypes. We concluded that food fortification with folic acid prevents NTDs but not other types of congenital anomalies.

Key words: folic acid; food fortification; fortified flour; neural tube defects; NTD; anencephaly; spina bifida; cephalocele; congenital anomalies; birth defects monitoring; South America

How to Cite this Article:
López-Camelo JS, Castilla EE, Orioli IM. 2010. Folic acid flour fortification: Impact on the frequencies of 52 congenital anomaly types in three South American countries.
Am J Med Genet Part A 152A:2444–2458.

INTRODUCTION

ECLAMC (Spanish acronym for Latin American Collaborative Study of Congenital Malformations) [Castilla and Orioli, 2004] regularly monitors the occurrence of congenital anomalies in South American countries since the late 1960s and early 1970s.

In South America, mandatory folic acid (FA) food fortification, with dosages aimed at the primary prevention of congenital anomalies, has been implemented in 3 of the 10 countries, starting in Chile in 2000, and followed by Argentina in 2003, and Brazil in 2004. However, fortification policies vary among these three countries; the estimated daily dose of FA is around 500 μg in Chile [Hertrampf et al., 2003], and Argentina [Calvo and Biglieri, 2008; Zabala et al., 2008], and half of that dose (264 μg) in Brazil [Ferreira and Giugliani, 2008].

The effectiveness of these FA fortification programs on the prevention of NTDs has already been analyzed by several

Grant sponsor: CONICET PEI, Argentina; Grant number: 6475-2007-2008; Grant sponsor: DECIT/CNPq, Brazil; Grant number: 408863/2006-4; Grant sponsor: MCT/CNPq, Brazil; Grant numbers: 573993/2008-4, 476978/2008-4, 308885/2006-6; Grant sponsor: FAPERJ, Brazil; Grant number: E-26/102.748/2008.
*Correspondence to:
Iêda M. Orioli, LMC, Departamento de Genética, Universidade Federal do Rio de Janeiro, Caixa Postal 68.011, 21944-970 Rio de Janeiro, Brazil.
E-mail: orioli@centroin.com.br
Published online 2 September 2010 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/ajmg.a.33479

investigators in Chile [Freire et al., 2000; Castilla et al., 2003; Hertrampf et al., 2003; López-Camelo et al., 2005; Corral et al., 2006; Nazer et al., 2007], Argentina [Calvo, 2008; Calvo and Biglieri, 2008; Zabala et al., 2008], and Brazil [Pacheco-Santos and Pereira, 2007; Pacheco et al., 2009]. However, to the authors' knowledge, no data have been published in South America on the effects of fortification for congenital anomalies other than NTDs, except for some subsets of the ECLAMC material presented in the present paper [Castilla et al., 2003; López-Camelo et al., 2005; Nazer et al., 2007].

Botto et al. [2006] evaluated surveillance data on major birth defects of population-based registries from Europe, North America, and Australia. They concluded that FA fortification appears to be effective in reducing NTDs, but the effect on other birth defects remains unclear. In a systematic review of the efficacy of FA fortification to decrease NTD prevalence, Leoncini and Mastroiacovo [2009] found inconclusive results from other parts of the world.

This is the third ECLAMC publication of an ongoing surveillance of FA and birth defects, albeit the first one with data from other countries besides Chile, and with samples large enough to allow the monitoring of birth defects other than those of the neural tube [Castilla et al., 2003; López-Camelo et al., 2005]. The present investigation tested the null hypothesis, assuming no significant reduction in the birth prevalence estimates of 52 selected types of congenital anomalies, between the pre- and post-FA fortification periods in Chile, Argentina, and Brazil.

METHODS

ECLAMC is a hospital-based, voluntary network dedicated to the research and monitoring of congenital anomalies in South America since 1967. It includes 114 reporting maternity hospitals distributed in all 10 South American countries, except Guianas [Castilla and Orioli, 2004]. Even though the ECLAMC database contains information since 1967 [Castilla and Orioli, 1985; Castilla et al., 1985], complete data on both live- and stillbirths, weighing 500 g or more (of approximately 22 or more gestational weeks), are only available since 1982. Therefore, the study period for this work was restricted to 1982–2007.

According to routine ECLAMC procedures, all consecutive live- and stillbirths occurring in participating hospitals were examined for major and minor congenital anomalies. Each malformed newborn infant was one-to-one matched to a control, defined as the immediately subsequent non-malformed, like-sexed livebirth occurring in the same hospital [Castilla and Orioli, 2004]. Mothers of cases and controls were interviewed postpartum regarding 50 risk factors, including environmental exposures. Case definitions were based on verbatim descriptions given by the reporting pediatricians at birth or within the first week of life. Quality control of data is performed manually for verbatim descriptions and automatically for other data. The proportion of ascertained births is heterogeneous among countries, varying from 1% of all births in Brazil to 10% in Argentina and Chile. Pregnancy terminations are not reported because they are illegal in all South American countries.

Throughout this paper, results from the three countries will be discussed in the chronological order they started FA fortification, namely, Chile, Argentina, and Brazil. The material from the three selected countries included a total of 3,347,559 live- and stillborn infants (weighing 500 g or more), of 77 hospitals: 596,704 from Chile (17 hospitals), 1,643,341 from Argentina (41 hospitals), and 1,107,514 from Brazil (19 hospitals).

Fifty-two of about 300 different congenital anomaly types were selected among the ascertained consecutive birth series, based on available sample size and required statistical power; they are the diagnoses routinely monitored and reported by ECLAMC to the International Clearinghouse for Birth Defects Surveillance and Research and defined according to its norms [ICBDMS, 1991]. A total of 138,778 infants had one or more congenital anomalies (4.15%), and 85,213 of these had one or more of the 52 selected congenital anomaly types (2.68%).

Special attention was given to congenital heart defects because of their potential association with periconceptional FA intake [Robbins et al., 2006; Ionescu-Ittu et al., 2009], and their morphological subtypes were grouped for analysis under two different criteria:

(1) According to the ECLAMC routine [Castilla and Orioli, 2004], five categories were considered: conotruncal (truncus arteriosus, pulmonary artery defect, transposition of great arteries, tetralogy of Fallot, aorta defects, pulmonary valvular defect, and other conotruncal anomalies), septal (atrial septal defect [ASD], ventricular septal defect [VSD], single ventricle, and atrioventricular septal defect), valvular (mitral and tricuspid defects), other severe heart defects (hypoplastic left heart, coarctation of aorta, and total anomalous venous return), and unspecified congenital heart defects.
(2) For comparison with the recently published observations on FA fortification in Quebec, Canada, by Ionescu-Ittu et al. [2009], three categories were considered: severe conotruncal (tetralogy of Fallot, transposition of great arteries, and truncus arteriosus), severe non-conotruncal (atrioventricular septal defect and single ventricle), and severe-total, including the five defects listed above.

In order to preserve diagnostic preciseness, collective categories, such as "other," or "unspecified" were excluded, except for congenital heart defect of unspecified type. Recognized syndromes were excluded as well, except for Down syndrome, which was split into two maternal age groups, 19 years or less and 35 years or older, to increase its etiological specificity [ICBDMS, 1991].

Each of the 52 congenital anomaly types was considered as isolated and total (isolated plus cases with other unrelated anomalies in the same infant), because the isolated forms are expected to be less heterogeneous from an etiopathogenetic standpoint. Even though some isolated cases could have been misclassified as associated because of the presence of minor or pathogenetically related anomalies, we preferred to consider them as such, with the purpose of increasing homogeneity of the isolated group. Infants with more than one anomaly were counted more than once, and therefore, the tables do not present total values.

Two obvious limitations of historical series in hospital-based registries, with "hospital" as the geographic observation unit, are the lack of continuity of each participating hospital, and the unequally biased selection of high-risk pregnancies. Thus, pre- versus post-fortification comparisons were adjusted by hospital of birth, by comparing pre- and post-fortification periods within each hospital, and by pooling the resulting Prevalence Rate Ratios (PRRs) by country, a method already applied in our two previous studies [Castilla et al., 2003; López-Camelo et al., 2005]. A PRR of 0.70 means a birth prevalence reduction of 0.30 (30%) for a specific congenital anomaly between the pre- and post-fortification periods.

To evaluate changes in birth prevalence for each anomaly type between pre-fortification (baseline), and post-fortification periods, we first estimated with a Poisson regression analysis if there was any secular linear trend before fortification began. The regression model was applied to each malformation in each country sub-sample, and the independent variables, temporal changes, both linear and quadratic, were entered into the model. The expected number of cases for the fortified period was then estimated by projection, and these values were compared with actually observed numbers of cases. The resulting estimator was the observed/expected ratio. The observed pre- and post-fortification periods for each country are presented in Table I. The triennium immediately prior to the birth of the first infants periconceptionally exposed to fortification was used for analysis of pre- versus post-fortification rates. The pre-fortification triennium included the 12 months after the date FA fortification started in each country. This 1 year estimate includes 3 months for the newly fortified flour to be made available to the public, plus 9 months of full term gestation. If fortified children were erroneously included in the pre-fortification period, because the time to make fortified flour available to the public was less than 3 months, this would increase the significance of our results, if we disprove the null hypothesis of no difference between pre- and post-fortification periods.

The estimator was the PRR, adjusted by hospital. We used the "metan" routine of Stata, v. 7.0, for a random effects model that assumes heterogeneous fortification among hospitals from the different countries. This routine produced the already mentioned prevalence estimates for each hospital, active during the two consecutive periods; an overall prevalence estimate using the Mantel–Haenszel test, which weights individual hospitals' sample sizes; and a χ^2 heterogeneity test for hospitals' risk ratios with $k-1$ degrees of freedom, where k is the number of active hospitals in both periods. The significance of the Mantel–Haenszel test was evaluated with a Z test, and the prevalence estimate confidence intervals were obtained by the Cornfield method.

According to Bonferroni's correction, the critical value of significance was set at $P < 0.00016$, due to the large number of comparisons, namely, 52 anomalies times two categories (isolated and total), times three country sub-samples (312 comparisons), for which 15 false positive comparisons were to be expected if a $P < 0.05$ limit was chosen.

Statistical power was estimated for rate reduction values between 20% and 50%, and different birth prevalence estimates of the anomalies (1/1,000, 1/2,000, and 1/3,000), taking into consideration the sample sizes of the pre- and post-fortification periods in each of the three countries (Table I).

With the purpose of increasing the precision of the estimated relative risks, the PRR was only calculated for anomalies with 10 or more registered cases in the pre-fortification period (Tables II and III).

TABLE I. Summarized Relevant Information on Folic Acid Fortification (FAF) for Each of the Three South American Countries

Characteristics	Chile	Argentina	Brazil
Date FAF policy regulation	10/09/99	08/22/02	12/13/02
Date FAF policy implementation	01/01/00	11/13/03	06/13/04
Estimated date of first FAF births	01/01/01	11/13/04	06/13/05
Pre-FAF period	1998–2000	2002–2004	2003–2006/2005[c]
Pre-FAF number of observed births	69,677	193,509	102,751
FAF period	2001–2003	2005–2007	2007/2005–2007[d]
FAF number of observed births	243,624	147,853	92,843
Regulation type	Ministry Act	Federal Law	Ministry Act
FAF Flour	Wheat	Wheat	Wheat and maize
FA concentration in flour (mg/kg)	2.2[a]	2.2[b]	1.5[e]
Estimated daily intake of flour per capita (g)	227[a]	221[b]	176[e]
FA daily dose (μg)	499	486	264
Population in July 2008 (in millions)	16	42	192
Annual births	244,000	685,000	3,000,000

Dates: mm/dd/yy.

[a]Calvo and Biglieri [2008].
[b]Zabala et al. [2008] for Argentina.
[c]2003–2006/2005: January 2003 to June 2005.
[d]2007/2005–2007: July 2005 to December 2007; data for FA daily dose calculation are from Hertrampf et al. [2003] for Chile.
[e]Ferreira and Giugliani [2008] for Brazil.

TABLE II. Folic Acid Fortification Effect on Birth Prevalence Rates (/10,000) of 52 Specific Types of Congenital Anomalies as Totals (Isolated Plus Associated Forms) in the Three South American Countries, Adjusted by Hospital

	Chile			Argentina			Brazil		
	PRR	95% CI	*P*	PRR	95% CI	*P*	PRR	95% CI	*P*
Omphalocele	0.95	0.56–1.62	0.856	1.34	0.92–1.96	0.782	1.00	0.62–1.61	0.994
Gastroschisis	0.80	0.40–1.61	0.531	1.60	1.06–2.41	0.023	1.29	0.91–1.82	0.156
Anencephaly	0.54	0.36–0.83	0.004	0.59	0.42–0.82	0.002	0.57	0.37–0.87	0.010
Spina bifida-cephalic	0.17	0.07–0.42	<0.0001	0.27	0.14–0.53	<0.0001	0.49	0.26–0.96	0.036
Spina bifida-caudal	0.55	0.37–0.81	0.002	0.75	0.57–0.99	0.044	1.12	0.62–2.02	0.703
Spina bifida-total	0.43	0.31–0.60	<0.0001	0.59	0.46–0.76	<0.0001	0.99	0.56–1.74	0.973
Hydrocephaly	1.06	0.79–1.47	0.715	1.15	0.93–1.42	0.189	0.84	0.68–1.03	0.096
Cephalocele	0.47	0.27–0.83	0.009	0.83	0.53–1.31	0.437	0.59	0.33–1.08	0.091
Microcephaly	1.20	0.66–2.20	0.541	1.25	0.83–1.27	0.288	0.88	0.58–1.32	0.530
An/microtia	1.15	0.78–1.70	0.490	0.83	0.59–1.17	0.291	1.14	0.81–1.75	0.586
CHD-conotruncal	1.30	0.94–1.39	0.111	0.69	0.42–1.07	0.063	1.03	0.71–1.49	0.872
CHD-septal	1.17	1.00–1.38	0.048	0.63	0.54–0.75	0.002	0.72	0.59–0.88	0.002
CHD-valvular	0.90	0.47–1.72	0.756	0.83	0.46–1.48	0.529	1.12	0.57–2.21	0.745
CHD-other severe	1.07	0.66–1.73	0.777	0.86	0.57–1.29	0.456	1.37	0.54–2.73	0.311
CHD-unspecified	1.12	0.67–1.88	0.658	1.60	0.78–3.28	0.200	0.63	0.40–0.99	0.045
CHD-severe-total (I-I)	1.28	0.95–1.73	0.098	0.66	0.50–96	0.028	0.77	0.51–1.17	0.226
CHD-severe-conotruncal (I-I)	1.46	0.99–2.26	0.056	0.85	0.56–1.29	0.446	1.14	0.70–1.83	0.601
CHD-severe-non-conotruncal (I-I)	1.02	0.66–1.60	0.902	0.57	0.35–0.92	0.021	0.27	0.11–0.69	0.004
Cleft palate only	1.22	0.73–2.06	0.441	1.43	1.04–1.48	0.027	0.68	0.46–1.04	0.077
Cleft lip ± cleft palate	0.97	0.74–1.27	0.819	0.79	0.61–1.03	0.081	1.20	0.91–1.57	0.196
Cleft lip only	0.81	0.46–1.43	0.462	0.97	0.63–1.51	0.906	1.15	0.70–1.89	0.573
Cleft lip and palate	0.99	0.72–1.33	0.929	0.81	0.62–1.07	0.129	1.21	0.87–1.69	0.245
Esophageal atresia	0.67	0.34–1.30	0.233	1.09	0.74–1.60	0.662	0.53	0.31–0.90	0.020
Duodenal atresia	1.02	0.44–2.32	0.959	1.06	0.53–2.10	0.867	0.92	0.45–1.86	0.815
Anal atresia	0.88	0.60–1.31	0.537	0.94	0.69–1.27	0.699	0.80	0.49–1.31	0.380
Ambiguous genitalia	—	—	—	1.07	0.63–1.83	0.803	1.34	0.79–2.27	0.283
Hypospadias-total	0.99	0.66–1.49	0.981	0.69	0.51–0.93	0.016	0.90	0.73–1.10	0.318
Hypospadias-distal	0.83	0.55–1.24	0.366	0.71	0.51–0.99	0.046	0.84	0.67–1.05	0.134
Hypospadias-proximal	—	—	—	0.63	0.55–1.25	0.314	1.06	0.56–2.11	0.872
Absent kidney/s	1.04	0.51–2.13	0.916	1.18	0.71–1.94	0.515	0.90	0.56–1.46	0.687
Polycystic kidneys	0.88	0.59–1.32	0.542	0.92	0.64–1.32	0.655	0.96	0.64–1.44	0.856
Hydronephrosis	1.47	0.92–2.34	0.106	1.30	0.95–1.78	0.103	1.13	0.74–1.73	0.559
Talipes equinovarus	1.03	0.75–1.43	0.840	0.96	0.80–1.15	0.639	0.94	0.80–1.10	0.433
Talipes calcaneovalgus	0.68	0.50–0.93	0.015	1.02	0.67–1.66	0.920	0.91	0.66–1.25	0.565
Polydactyly-post-axial	0.92	0.71–1.21	0.575	0.81	0.66–1.02	0.071	0.87	0.67–1.14	0.329
Polydactyly-pre-axial	0.91	0.60–1.39	0.671	1.15	0.80–1.66	0.451	1.21	0.59–1.48	0.597
Polydactyly-others	—	—	—	0.77	0.32–1.87	0.573	1.08	0.42–1.77	0.865
Syndactyly-toes 2–3	0.55	0.30–0.99	0.047	1.66	0.77–3.59	0.197	1.53	0.78–3.05	0.216
Syndactyly-other types	1.28	0.77–2.11	0.327	1.32	0.89–1.95	0.169	1.12	0.71–1.78	0.621
LRD-TT: amputation	0.83	0.47–1.49	0.544	0.61	0.39–0.98	0.041	0.41	0.24–0.70	<0.0001
LRD-TTH: hypoplasia	—	—	—	0.90	0.40–2.02	0.803	0.67	0.26–1.74	0.413
LRD-pre-axial	—	—	—	1.00	0.52–1.91	0.993	0.64	0.30–1.35	0.241
Hip-subluxation	0.58	0.32–1.06	0.077	0.75	0.46–1.22	0.250	0.80	0.46–1.39	0.422
Hip-dislocation	—	—	—	1.06	0.50–1.22	0.881	—	—	—
Arthrogryposis	0.66	0.36–1.22	0.183	0.43	0.24–0.78	0.005	0.46	0.22–0.97	0.041
Diaphragmatic hernia	0.75	0.49–1.14	0.183	1.35	0.84–2.18	0.211	0.84	0.44–1.59	0.596
Abdominal muscle deficiency	—	—	—	0.72	0.31–1.68	0.449	0.49	0.21–1.16	0.106
Pectoralis hypoplasia	—	—	—	1.09	0.44–2.72	0.850	—	—	—
Skin ring constriction	—	—	—	0.43	0.19–1.00	0.049	0.82	0.37–1.77	0.608
Down total	1.09	0.91–1.32	0.326	1.01	0.86–1.20	0.859	0.81	0.65–1.02	0.069
Down syndrome ≤19 YMA	0.55	0.29–1.03	0.064	2.00	1.10–3.64	0.023	0.85	0.38–1.84	0.666
Down syndrome ≥35 YMA	1.33	1.03–1.73	0.027	1.05	0.82–1.34	0.705	0.88	0.64–1.20	0.420

PRR, prevalence rate ratio; spina bifida-cephalic, cervical, thoracic; spina bifida-caudal, lumbar, sacral; CHD, congenital heart disease; (I-I), CHD grouped as Ionescu-Ittu et al. [2009]; hypospadias-distal, balanic, balano-prepucial; hypospadias-proximal, penile, scrotal, perineal; LRD, limb reduction defect; TT, transverse terminal; TTH, transverse terminal hypoplasia (includes brachydactyly); YMA, years of maternal age.

TABLE III. Folic Acid Fortification Effect on Birth Prevalence Rates (/10,000) of 52 Specific Types of Congenital Anomalies in Their Isolated Forms in the Three South American Countries, Adjusted by Hospital

	Chile			Argentina			Brazil		
	PRR	95% CI	*P*	PRR	95% CI	*P*	PRR	95% CI	*P*
Omphalocele	—	—	—	0.84	0.51–1.40	0.517	0.80	0.48–1.36	0.420
Gastroschisis	0.54	0.25–1.170	0.117	1.40	0.89–1.94	0.112	1.09	0.75–1.58	0.634
Anencephaly	0.36	0.22–0.61	<0.0001	0.51	0.36–0.73	<0.0001	0.47	0.32–0.68	<0.0001
Spina bifida-cephalic	0.06	0.01–0.24	<0.0001	0.21	0.10–0.47	<0.0001	0.35	0.14–0.85	<0.0001
Spina bifida-caudal	0.38	0.23–0.62	<0.0001	0.55	0.38–0.80	0.002	0.57	0.37–0.88	0.012
Spina bifida-total	0.30	0.19–0.46	<0.0001	0.42	0.30–0.59	<0.0001	0.52	0.35–0.76	<0.0001
Hydrocephaly	1.09	0.70–1.68	0.688	0.88	0.64–1.19	0.416	0.64	0.47–0.85	0.021
Cephalocele	0.22	0.10–0.45	<0.0001	0.59	0.32–1.07	0.086	0.44	0.23–0.85	0.014
Microcephaly	1.64	0.91–2.95	0.098	0.91	0.46–1.79	0.784	0.80	0.47–1.37	0.412
An/microtia	0.96	0.61–1.50	0.863	0.50	0.30–0.84	0.046	0.84	0.39–1.80	0.651
CHD-conotruncal	1.33	0.90–1.95	0.150	0.57	0.38–0.84	0.006	0.80	0.51–1.26	0.352
CHD-septal	1.14	0.92–1.40	0.227	0.56	0.44–0.70	0.003	0.69	0.57–0.91	0.009
CHD-valvular	—	—	—	1.10	0.56–2.15	0.783	0.94	0.41–2.14	0.883
CHD-other severe	0.76	0.45–1.31	0.333	1.01	0.65–1.59	0.943	1.35	0.63–2.88	0.436
CHD-unspecified	0.57	0.29–1.08	0.085	1.10	0.36–3.39	0.871	0.62	0.34–1.10	0.104
CHD-severe-total (I-I)	1.46	0.95–2.23	0.081	0.43	0.27–0.68	0.003	0.84	0.48–1.47	0.540
CHD-severe-conotruncal (I-I)	1.57	0.97–2.55	0.067	0.66	0.31–1.01	0.059	0.83	0.47–1.47	0.523
CHD-severe-non-conotruncal (I-I)	—	—	—	0.23	0.10–0.55	0.004	—	—	—
Cleft palate only	1.84	0.96–3.55	0.068	0.87	0.48–1.54	0.614	0.42	0.20–0.88	0.021
Cleft lip ± cleft palate	0.76	0.55–1.04	0.087	0.67	0.52–0.88	0.003	0.98	0.70–1.41	0.939
Cleft lip only	0.70	0.37–1.36	0.301	0.66	0.40–1.08	0.099	1.06	0.60–1.88	0.841
Cleft lip and palate	0.74	0.52–1.06	0.099	0.64	0.47–0.87	0.004	0.91	0.58–1.44	0.698
Esophageal atresia	—	—	—	0.82	0.49–1.39	0.470	0.36	0.16–0.82	0.015
Duodenal atresia	—	—	—	—	—	—	—	—	—
Anal atresia	0.76	0.39–1.48	0.424	0.73	0.45–1.20	0.219	—	—	—
Ambiguous genitalia	—	—	—	—	—	—	0.40	0.15–1.34	0.234
Hypospadias-total	0.78	0.56–1.10	0.157	0.66	0.47–0.90	0.021	0.80	0.63–0.99	0.040
Hypospadias-distal	0.73	0.52–1.03	0.077	0.69	0.49–0.99	0.035	0.76	0.60–0.99	0.026
Hypospadias-proximal	—	—	—	—	—	—	0.91	0.41–2.02	0.830
Absent kidney/s	—	—	—	1.22	0.67–2.22	0.505	0.60	0.28–1.29	0.191
Polycystic kidneys	1.01	0.72–1.20	0.204	0.84	0.55–1.32	0.455	0.71	0.42–1.20	0.204
Hydronephrosis	0.51	0.22–1.16	0.109	0.77	0.50–1.17	0.224	0.60	0.20–1.62	0.291
Talipes equinovarus	1.00	0.72–1.25	0.700	0.99	0.68–1.05	0.117	0.95	0.72–1.25	0.700
Talipes calcaneovalgus	0.58	0.42–0.80	0.003	0.75	0.45–1.24	0.265	0.60	0.42–0.80	0.002
Polydactyly-post-axial	1.04	0.70–1.98	0.693	0.70	0.54–0.90	0.005	0.94	0.70–1.27	0.693
Polydactyly-pre-axial	1.06	0.62–1.80	0.841	0.74	0.50–1.12	0.161	1.06	0.62–1.80	0.841
Polydactyly-others	—	—	—	0.50	0.20–1.32	0.163			
Syndactyly-toes 2–3	—	—	—	—	—	—	1.41	0.73–2.72	0.299
Syndactyly-other types	—	—	—	0.45	0.13–1.60	0.220	3.50	0.14–6.03	0.442
LRD-TT: amputation	—	—	—	0.66	0.37–1.16	0.146	0.34	0.15–1.62	0.432
LRD-TTH: hypoplasia	—	—	—	—	—	—	—	—	—
LRD-pre-axial	—	—	—	—	—	—	—	—	—
Hip-subluxation	0.42	0.30–0.60	<0.0001	0.40	0.23–0.70	<0.0001	0.71	0.56–0.90	0.005
Hip-dislocation	—	—	—	—	—	—	—	—	—
Arthrogryposis	—	—	—	0.30	0.10–1.05	0.081	—	—	—
Diaphragmatic hernia	0.80	0.49–1.29	0.343	1.04	0.68–1.60	0.850	0.54	0.30–0.98	0.053
Abdominal muscle deficiency	—	—	—	—	—	—	—	—	—
Pectoralis hypoplasia	—	—	—	—	—	—	—	—	—
Skin ring constriction	—	—	—	—	—	—	—	—	—

PRR, prevalence rate ratio; spina bifida-cephalic, cervical, thoracic; spina bifida-caudal, lumbar, sacral; CHD, congenital heart disease; (I-I), CHD grouped as Ionescu-Ittu et al. [2009]; hypospadias-distal, balanic, balano-prepucial; hypospadias-proximal, penile, scrotal, perineal; LRD, limb reduction defect; TT, transverse terminal; TTH, transverse terminal hypoplasia (includes brachydactyly); YMA, years of maternal age.

RESULTS

Statistical Power

With the available sample sizes for both periods, in Chile, a power greater than 80% was obtained to detect a minimal reduction of 40% for anomalies with birth prevalence estimates of 1/1,000, and of 50% for those with birth prevalence estimates of 1/2,000. In Argentina, a power greater than 80% was obtained to detect a minimal reduction of 30% for anomalies with birth prevalence estimates of 1/1,000, of 40% for 1/2,000, and of 50% for those of approximately 1/3,000. In Brazil, the sample sizes rendered a power greater than 80% to detect a minimal reduction of 40% for anomalies with prevalence estimates of 1/1,000, and of 50% for those of approximately 1/2,000.

Secular Trends Before Fortification

Secular trends during the pre-fortification period were estimated for each of the 52 congenital anomaly types, grouped as totals (isolated plus associated) (data not shown—available from the corresponding author).

In Chile, significantly rising secular trends ($P < 0.0001$) were observed for gastroschisis, Down syndrome with mothers of 35 years or older, and total Down syndrome; and decreasing trends for congenital heart defects of unspecified type, and subluxation of the hip.

In Argentina, significantly rising secular trends ($P < 0.0001$) were observed for gastroschisis, spina bifida (cephalic, caudal, and total), hydrocephaly, cephalocele, cleft lip and palate, anal atresia, absent kidneys, polycystic kidneys, hydronephrosis, pre-axial polydactyly, arthrogryposis, and diaphragmatic hernia. Decreasing trends were significant for congenital heart defects of unspecified type, subluxation of the hip, and true dislocation of the hip.

In Brazil, significantly rising secular trends ($P < 0.0001$) were observed for omphalocele, gastroschisis, spina bifida (cephalic, caudal, and total), hydrocephaly, cephalocele, microcephaly, an/microtia, cleft palate, esophageal atresia, duodenal atresia, ambiguous genitalia, hypospadias (proximal and total), absent kidneys, polycystic kidneys, hydronephrosis, pes equinovarus, talipes calcaneovalgus, post-axial polydactyly, transverse limb deficiency, pre-axial limb defect, true dislocation of the hip, arthrogryposis, diaphragmatic hernia, abdominal muscle deficiency, skin ring constriction (as seen in the amniotic band sequence), and Down syndrome, with maternal age of 35 years or above and total. No significantly decreasing trend was observed for any anomaly type.

The observed/expected ratios (not shown), with expected values adjusted by projected secular trends, confirmed the results of the intra-hospital comparison approach for a significance level under 0.0001 (Table II).

Prevalence Rate Ratios (PRRs)

Significant reductions in birth prevalence estimates after fortification were observed for eight of the 52 investigated congenital anomaly types, in their total (Table II), and isolated forms (Table III). The observed numbers by malformation are shown in Appendix A for Chile, in Appendix B for Argentina, and in Appendix C for Brazil.

For isolated anencephaly, significant ($P < 0.0001$) PRRs were registered in all three investigated countries. For isolated cephalic (cervical-thoracic) spina bifida, significant ($P < 0.0001$) PRRs were registered in all three investigated countries, and for the total, in Chile and Argentina. For isolated caudal (lumbo-sacral) spina bifida, significant ($P < 0.0001$) PRRs were registered only in Chile, and for the total, in none of the three country sub-samples. For isolated total spina bifida (cephalic, caudal, and unspecified levels), significant ($P < 0.0001$) PRRs were registered in all three investigated countries, and for the total, in Chile and Argentina. For cephalocele, significant PRRs were only observed for its isolated form in Chile.

For septal heart defects, no significant PRRs were observed. However, marginal significance was registered for their isolated forms in Argentina and Brazil. For transverse limb deficiency, the PRR was significant only for its total form in Brazil. For subluxation of the hip, the PRR was only significant for its isolated form in Chile and Argentina; in Brazil the difference was of marginal significance.

Table IV shows the birth prevalence estimates for isolated and total forms of NTDs, during the pre- and post-fortification periods,

TABLE IV. Birth Prevalence Estimates for Neural Tube Defects (Isolated and Total) in Pre-Fortification and Post-Fortification Periods

	Chile				Argentina				Brazil			
	Pre-fortification		Post-fortification		Pre-fortification		Post-fortification		Pre-fortification		Post-fortification	
	Isolated	Total	Isolated	Total	Isolated	Total	Isolated	Total	Isolated	Total	Isolated	Total
Anencephaly	0.52	0.63	0.26	0.37	0.69	0.86	0.29	0.37	0.90	1.12	0.45	0.69
Spina bifida-total	0.73	1.02	0.24	0.46	0.82	1.27	0.33	0.66	0.86	1.45	0.69	1.42
Spina bifida-cephalic	0.16	0.26	0.01	0.05	0.24	0.37	0.02	0.05	0.18	0.33	0.06	0.14
Spina bifida-caudal	0.55	0.72	0.21	0.38	0.57	0.88	0.30	0.60	0.62	1.04	0.56	1.23
Cephalocele	0.26	0.33	0.09	0.18	0.21	0.32	0.10	0.20	0.31	0.57	0.12	0.32

BP, birth prevalence/1,000 births.
Pre-fortification period in Chile 1998–2000, post-fortification period 2001–2007.
Pre-fortification period in Argentina 2002–2004, post-fortification period 2005–2007.
Pre-fortification period in Brazil 2003–2006/2005, post-fortification period 2007/2005–2007.

expressed per 1,000 births, in order to facilitate comparisons with previously published results from Canada [De Wals et al., 2007].

DISCUSSION

The present study involves three Latin American countries where FA fortification has been implemented, 52 congenital anomalies, and the 1982–2007 period, and it partially overlaps with three previous studies with ECLAMC material: Castilla et al. [2003] who dealt with five Latin American countries (with fortification only in Chile), three types of congenital anomalies (NTDs, oral clefts, and Down syndrome), and the 1999–2001 period; López-Camelo et al. [2005] who dealt only with material from Chile, two types of congenital anomalies (spina bifida and anencephaly), and the 1982–2002 period; Nazer et al. [2007] who analyzed 14 Chilean hospitals, 24 congenital anomalies, and the 1995–1999 period. The present study corroborates the reduction of NTDs after FA fortification observed in the three previous studies, but not the reduction of diaphragmatic hernia observed by Nazer et al. [2007], probably because their data were not corrected by secular trends.

Limitations and Strengths

As with any ecological study, our study can only suggest cause-effect associations, since interactions with many other uncorrected or partially adjusted factors are very likely to occur, such as the increasing number of pregnancy terminations for anencephaly, mainly in Brazil, less in Argentina, and much less in Chile. Even though pregnancy terminations are illegal in all three countries, individual judge permissions can overrule the law, and they are becoming more common in Brazil, less in Argentina, while still nonexistent in Chile.

As in any hospital-based study, the investigated consecutive births were non-random, as well as biased, small, and non-representative samples of a universe of births, adding up to more than four million per year. Even though the crude observed/expected values were adjusted by hospital, this correction might have been incomplete.

Despite the 80% power to detect a 30–50% decrease in the rates of the selected anomalies, we may have lacked sufficient power to identify more subtle changes that could be expected for other birth defects.

We did not take into account possible differences in the use of supplements during the pre- and post-fortification periods. However, this was not seen as a limitation, because in these countries, supplements are usually prescribed after pregnancy has been detected, and, therefore, have no influence on the prevention of NTDs [Botto et al., 2006].

The strengths of the present work include availability of data from the years prior to fortification; large sample sizes for the baseline, as well as for the observation periods; detailed clinical descriptions of congenital anomalies (verbatim instead of codes), and the unbiased project design and data collection process, aimed at the study of causal risk factors for birth defects in general, instead of specifically evaluating a protective environmental factor, such as FA.

Since most congenital anomalies are heterogeneous from an etiological standpoint, we preferred to delineate them into presumably more homogeneous sub-phenotypes, and the detailed clinical descriptions available in the ECLAMC database allows for such precise delineations (e.g., "incomplete, two-thirds, left-sided, cleft of the lip, with ipsilateral gum notch, and normal hard and soft palate"). However, an intermediate degree of splitting was used, in order not to break down the material into diagnostic units too small to be evaluated.

Folic Acid Fortification Effect on NTDs: Types and Sub-Types

In the present material, the occurrence of total spina bifida (cephalic, caudal, and unspecified levels) decreased significantly and consistently in all three countries, except for the total form (isolated plus associated) in Brazil, possibly related to the smaller sample size. Isolated anencephaly also decreased in the three countries, while the reduction of cephalocele was only significant in its isolated form in Chile.

The stronger effect of FA fortification on spina bifida than on anencephaly and cephalocele has already been reported in the Chilean sub-sample [López-Camelo et al., 2005], as well as in other parts of the world, such as the United States [Williams et al., 2002], Canada [De Wals et al., 2007], and South Africa [Sayed et al., 2008]. In our case series, open spina bifida is more accurately diagnosed than the other two NTDs. Some infants who were registered as having anencephaly or cephalocele actually have acrania or other disruptive defects of the cranial vault, not due to a failure of the neural tube closure process, and this is particularly expected to occur in the associated forms, such as those due to constriction bands or other exogenous factors. Our results clearly support the greater sensitivity of the rarer and more severe, higher level (cephalic) spina bifida aperta to FA prevention, described by De Wals et al. [2008] with Canadian data, while to our knowledge no other observations on NTD subtypes have been published. In conclusion, these observations suggest that isolated cephalic spina bifida is the most sensitive defect to FA, not only among NTDs, but among all congenital anomalies.

The direct correlation between baseline birth prevalence estimates and prevalence reduction rates after FA fortification, reported by De Wals et al. [2007] in different provinces in Canada, was also observed in the three South American sub-samples reported here. Unlike the Canadian material, our three regions correspond to three countries with different FA doses, length of fortified period, and strategies of fortification, and of which Chile has the largest post-fortification sample, and for the longest time period: 243,624 births during 7 years. Considering the isolated forms, the reduction rate of anencephaly attained in Chile was 50%, from 0.52 to 0.26 per 1,000, while in Argentina the reduction was 58%, from 0.69 to 0.29 per 1,000. The birth prevalence estimates, higher in Argentina than in Chile before fortification, leveled off after fortification (0.29 and 0.26, respectively). Assuming no interaction with other variables, this observation suggests that Chile has already reached the maximum reduction (Table IV).

As shown in Table I, the fortification policy in Brazil differs from those in Chile and Argentina, with half of the concentration of FA and a lower estimated consumption of wheat-flour bread than the other two countries. However, a careful literature review [Leoncini and Mastroiacovo, 2009] revealed no correlation between population blood folate levels and birth prevalence estimates of NTDs. Thus, the explanation for the observed differences among countries could be more complex than expected. For instance, different responses to FA fortification among different races/ethnicities cannot be discarded. Such ethnic differences have been reported for Blacks in the USA [Williams et al., 2005], as well as for the aboriginal groups of Australia [Bower et al., 2009], and despite their geographic vicinity, the three countries considered in our study have large ethnic admixture differences [Wang et al., 2008]. In Brazil, the significant reduction in the birth prevalence rate of anencephaly, but not of spina bifida, strongly suggests the coincidental effect of pregnancy terminations, as mentioned above.

Folic Acid Fortification Effect on Other Defects

A number of reports on birth prevalence reductions of several congenital anomaly types, other than NTDs, after FA fortification, have been published [Simmons et al., 2004; Canfield et al., 2005; Robbins et al., 2006; Ionescu-Ittu et al., 2009]. In the present study, inconclusive results were obtained for three of the 47 non-NTD defects, namely, septal defects, transverse terminal limb defects, and subluxation of the hip.

Among the sub-types of congenital heart defects, isolated septal defects decreased, although without statistical significance, in the sub-samples of Argentina and Brazil, while the reduction of severe heart defects reported by Ionescu-Ittu et al. [2009] in Canada, was not observed in our material. Canfield et al. [2005] found a post-fortification reduction in transposition of the great arteries; however, this reduction was not observed by Robbins et al. [2006], in a study based on hospital records.

The observed prevalence reduction of transverse terminal limb defects was only significant for its total form and in the Brazilian sub-sample. Decreasing rates of limb defects after FA fortification were already published by other authors in the US [Simmons et al., 2004; Canfield et al., 2005; Robbins et al., 2006], without specification of the limb defect sub-types.

The prevalence reduction of subluxation of the hip, observed after fortification in Chile, Argentina, and with marginal significance in Brazil, has not been reported in the literature, nor has this defect been previously associated with FA.

Even though data are available in the ECLAMC database, the effects of FA fortification on other adverse pregnancy outcomes, such as preterm delivery, low birth weight, and twinning were considered out of the scope of this work, mainly because of the many intervening confounders. For instance, Nazer et al. [2006] reported increased twinning rates in an ECLAMC sample from Chile, in coincidence with the beginning of FA fortification. However, other putative factors were not considered, such as the increasing availability of assisted reproductive technologies, shown to be largely responsible for the raising multiple birth rates worldwide [Vollset et al., 2005].

Reliability of These Observations

Many congenital anomalies within the ECLAMC sample have shown rising secular trends during the pre- and post-fortification periods, mainly due to better ascertainment (e.g., congenital heart defects), as well as for other unknown reasons (e.g., gastroschisis). The intra-hospital comparison approach, as well as the projection of the observed trend on the expected post-fortification prevalence, and the short interval between the two compared periods were expected to reduce this limitation, although not to eliminate it entirely. Thus, some spurious positive associations are expected. Nonetheless, other criteria could help understand the actual meaning of the crude results obtained, namely, inter-country consistency, coincidental published findings, marginal statistical significance, reduction effect greater in isolated than in associated forms, and biologic plausibility.

Under this scope, all of our findings on NTDs are consistent and reliable, while the relevance of those on the remaining three observed prevalence reductions (of septal defects, transverse terminal limb defects, and subluxation of the hip) is at least doubtful. The rate reduction of septal defects after fortification did not reach the pre-established critical level of significance, not even in Chile, with the largest fortified sample size and longest fortification period. However, the fact that this effect only occurred in the isolated form of the anomaly provides some biological support. No reasonable explanation could be found for the rate reduction of transverse limb deficiencies in just one country, only for the total and not for the isolated form; it is however consistent with previously published observations on limb defects in general. Although the rate reduction of subluxation of the hip was found in all three sub-samples, the low observational value of this diagnosis at birth, that is, the low concordance rate of the diagnosis by different observers, seriously affects its relevance.

Congenital Anomalies Without a Significant Reduction in Prevalence in the Present Study

Previous studies have shown reductions in birth prevalence following FA fortification for oral clefts in general [Simmons et al., 2004], and for cleft palate only [Canfield et al., 2005], while negative results were reported by Robbins et al. [2006], and Sayed et al. [2008], as well as by us in a previous publication with preliminary data from Chile [Castilla et al., 2003].

For omphalocele, a significant reduction was reported by Canfield et al. [2005], and suggested by Simmons et al. [2004]. For Down syndrome, a non-significant reduction was observed by Simmons et al. [2004] in Arkansas, and a negative result by us, in our previous report with Chilean data [Castilla et al., 2003]. For renal agenesis and pyloric stenosis, reductions were only reported by Canfield et al. [2005]. For diaphragmatic hernia, a significant reduction was reported in a maternity hospital from Chile, whose data are included in the present work [Nazer et al., 2007].

The discussion of the present results on food fortification with FA excludes observations made after FA supplementation because of the large, and sometimes not well understood differences between these two different intervention types: supplementation and fortification [Botto et al., 1999].

Recommendations for Developing Countries

Nationwide and mandatory FA food fortification strategies are recommended for the primary prevention of NTDs in transitional developing countries, such as most of the Latin American ones. This recommendation is mainly based on the high frequency of unintended pregnancies [Gadow et al., 1998], making periconceptional supplementation ineffective, as well as on the high cost-benefit ratio of a national fortification program, shown in Chile [Hertrampf and Cortés, 2008], and South Africa [Sayed et al., 2008].

As in any other large health intervention program, the larger the country, the more complex the FA fortification program organization. However, results from small or medium-sized countries, such as Costa Rica [Chen and Rivera, 2004] and Chile [Hertrampf and Cortés, 2008; present work], as well as from large and heterogeneous countries, such as Brazil or Argentina analyzed here, were conclusive about the effectiveness of the FA flour fortification program on the prevention of NTDs.

REFERENCES

Botto LD, Moore CA, Khoury MJ, Erickson JD. 1999. Neural-tube defects. N Engl J Med 341:1509–1519.

Botto LD, Lisi A, Bower C, Canfield MA, Dattani N, De Vigan C, De Walle H, Erickson DJ, Halliday J, Irgens LM, Lowry RB, McDonnell R, Metneki J, Poetzsch S, Ritvanen A, Robert-Gnansia E, Siffel C, Stoll C, Mastroiacovo P. 2006. Trends of selected malformations in relation to folic acid recommendations and fortification: An international assessment. Birth Defects Res Part A 76:693–705.

Bower C, D'Antoine H, Stanley FJ. 2009. Neural tube defects in Australia: Trends in encephaloceles and other neural tube defects before and after promotion of folic acid supplementation and voluntary food fortification. Birth Defects Res A Clin Mol Teratol 85:269–273.

Calvo EB. 2008. Folic acid fortification and neural tube defects (Spanish). Arch Argent Pediatr 106:291–292.

Calvo EB, Biglieri A. 2008. Impact of folic acid fortification on women nutritional status and on the prevalence of neural tube defects (Spanish). Arch Argent Pediatr 106:492–498.

Canfield MA, Collins JS, Botto LD, Williams LJ, Mai CT, Kirby RS, Pearson K, Devine O, Mulinare J, National Birth Defects Prevention Network. 2005. Changes in the birth prevalence of selected birth defects after grain fortification with folic acid in the United States: Findings from a multistate population-based study. Birth Defects Res A Clin Mol Teratol 73:679–689.

Castilla EE, Orioli IM. 1985. Epidemiology of neural tube defects in South America. Am J Med Genet 22:695–702.

Castilla EE, Orioli IM. 2004. ECLAMC: The Latin American Collaborative Study of Congenital Malformations. Comm Genet 7:76–94.

Castilla EE, Orioli IM, López-Camelo JS. 1985. On monitoring the multiply malformed infant-I: Case-finding, case-recording, and data handling in a Latin-American program. Am J Med Genet 22:717–725.

Castilla EE, Orioli IM, López-Camelo JS, Dutra MG, Nazer-Herrera J. 2003. Preliminary data on changes in neural tube defect prevalence rates after folic acid fortification in South America. Am J Med Genet Part A 123A:123–128.

Chen LT, Rivera MA. 2004. The Costa Rican experience: Reduction of neural tube defects following food fortification programs. Nutr Rev 62:S40–S43.

Corral SE, Moreno SR, Perez GG, Ojeda BME, Valenzuela GH, Reascos MM, Sepulveda LW. 2006. Effect of flour folic acid fortification on the incidence of cranio-encephalic congenital defects. Rev Med Chile 134: 1129–1134.

De Wals P, Tairou F, Van Allen MI, Uh SH, Lowry RB, Sibbald B, Evans JA, Van den Hof MC, Zimmer P, Crowley M, Fernandez B, Lee NS, Niyonsenga T. 2007. Reduction in neural-tube defects after folic acid fortification in Canada. N Engl J Med 357:135–142.

De Wals P, Tairou F, Van Allen MI, Lowry RB, Evans JA, Van den Hof MC, Crowley M, Uh SH, Zimmer P, Sibbald B, Fernandez B, Lee NS, Niyonsenga T. 2008. Spina bifida before and after folic acid fortification in Canada. Birth Defects Res A Clin Mol Teratol 82:622–626.

Ferreira AFS, Giugliani R. 2008. Consumption of folic-acid-fortified flour and folate-rich foods among women at reproductive age in South Brazil. Community Genet 11:179–184.

Freire WB, Hertrampf E, Cortés F. 2000. Effect of folic acid fortification in Chile: Preliminary results. Eur J Pediatr Surg 10:42–43.

Gadow EC, Paz JE, Lopez-Camelo JS, Dutra MG, Queenan JT, Simpson JL, Jennings VH, Castilla EE. 1998. Unintended pregnancies in women delivering at 18 South American hospitals. Hum Reprod 13:1991–1995.

Hertrampf E, Cortés F. 2008. National food-fortification program with folic acid in Chile. Food Nutr Bull 29:S231–S237.

Hertrampf E, Cortés F, Erickson JD, Cayazzo M, Freire W, Bailey LB, Howson C, Kauwell GPA, Pfeiffer C. 2003. Consumption of folic acid-fortified bread improves folate status in women of reproductive age in Chile. J Nutr 133:3166–3169.

ICBDMS. 1991. Congenital malformations worldwide. Amsterdam: Elsevier. pp 41, 47, 53.

Ionescu-Ittu R, Marelli AJ, Mackie AS, Pilote L. 2009. Prevalence of severe congenital heart disease after folic acid fortification of grain products: Time trend analysis in Quebec, Canada. Br Med J 338:b1673.

Leoncini E, Mastroiacovo P. 2009. Systematic review of efficacy of folic acid fortification to decrease the prevalence of neural tube defects {Italian}. Document available on request from Alessandra Lisi International Centre on Birth Defects (ICBD), Roma, Italy. 2009. icbd@icbd.org

López-Camelo JS, Orioli IM, Dutra MG, Nazer-Herrera J, Rivera N, Ojeda ME, Canessa A, Wettig E, Fontannaz AM, Mellado C, Castilla EE. 2005. Reduction of birth prevalence rates of neural tube defects after folic acid fortification in Chile. Am J Med Genet Part A 135A:120–125.

Nazer HJ, Aguila RA, Cifuentes L. 2006. The frequency of twin pregnancies increased in a Chilean hospital associated with periconceptional flour folic acid supplementation (Spanish). Rev Med Chile 134:48–52.

Nazer HJ, Cifuentes OL, Aguila RA, Juárez HME, Cid RMP, Godoy VML, García AK, Melibosky RF. 2007. Effects of folic acid fortification in the rates of malformations at birth in Chile. Rev Med Chil 135:198–204.

Pacheco SS, Braga C, de Souza AI, Figueiroa JN. 2009. Effects of folic acid fortification on the prevalence of neural tube defects (Portuguese). Rev Saude Publica 43:565–571.

Pacheco-Santos LM, Pereira MZ. 2007. The effect of folic acid fortification on the reduction of neural tube defects. Cad Saúde Pública, Rio de Janeiro 23:17–24.

Robbins JM, Tilford JM, Bird TM, Cleves MA, Reading JA, Hobbs CA. 2006. Hospitalizations of newborns with folate-sensitive birth defects before and after fortification of foods with folic acid. Pediatrics 118:906–915 [Erratum in Pediatrics 118: 2608].

Sayed AR, Bourne D, Pattinson R, Nixon J, Henderson B. 2008. Decline in the prevalence of neural tube defects following folic acid fortification and its cost-benefit in South Africa. Birth Defects Res A Clin Mol Teratol 82:211–216.

Simmons CJ, Mosley BS, Fulton-Bond CA, Hobbs CA. 2004. Birth defects in Arkansas: Is folic acid fortification making a difference? Birth Defects Res A Clin Mol Teratol 70:559–564.

Vollset SE, Gjessing HK, Tandberg A, Rønning T, Irgens LM, Baste V, Nilsen RM, Daltveit AK. 2005. Folate supplementation and twin pregnancies. Epidemiology 16:201–205.

Wang S, Ray N, Rojas W, Parra MV, Bedoya G, Gallo C, Poletti G, Mazzotti G, Hill K, Hurtado AM, Camrena B, Nicolini H, Klitz W, Barrantes R, Molina JA, Freimer NB, Bortolini MC, Salzano FM, Petzl-Erler ML, Tsuneto LT, Dipierri JE, Alfaro EL, Bailliet G, Bianchi NO, Llop E, Rothhammer F, Excoffier L, Ruiz-Linares A. 2008. Geographic patterns of genome admixture in Latin American Mestizos. PLoS Genet 4:e1000037.

Williams LJ, Mai CT, Edmonds LD, Shaw GM, Kirby RS, Hobbs CA, Sever LE, Miller LA, Meaney FJ, Levitt M. 2002. Prevalence of spina bifida and anencephaly during the transition to mandatory folic acid fortification in the United States. Teratology 66:33–39.

Williams LJ, Rasmussen SA, Flores A, Kirby RS, Edmonds LD. 2005. Decline in the prevalence of spina bifida and anencephaly by race/ethnicity: 1995–2002. Pediatrics 116:580–586.

Zabala R, Waisman I, Corelli M, Tobler B, Bonora L, Cappato F, Cardetti M, Cervera M, Chepparo C, Costero A, Demarco R, Dieser P, Domínguez A, Dutto R, Encinas ME, Fiol AL, Gatica C, Giordano MA, González A, González D, Grassi ML, Machi C, Martínez A, Colombres R, Meersohn M, Merlo M, Miranda D, Nanzer J, Núñez JM, Pedano L, Pérez Pazo A, Puscama A, Ramos ER, Rosso J, Rubio A, Santinelli I, Sfreddo A, Silenzi G, Sobral A, Trigo M, Tuninetti B, Urrea S, Zabala R. 2008. Folic acid for neural tube defects prevention: Consumption and information in fertil-age women in Centro Cuyo Region (Spanish). Arch Argent Pediatr 106:295–301.

APPENDIX A. Number of cases by specific congenital anomaly types in their isolated (Iso) and total (isolated plus associated) forms, by year of birth, in Chile.

	Period (years)											
	1982–1987 (86,168[a])		1988–1992 (113,095[a])		1993–1997 (84,140[a])		1998–2001 (69,677[a])		2002–2004 (144,950[a])		2005–2007 (98,674[a])	
	Iso	Total	Iso	Total	Iso	Total	Iso	Total	Iso	Total	Iso	Total
Omphalocele	10	19	13	35	10	22	8	19	11	38	7	28
Gastroschisis	2	3	3	6	9	9	12	14	19	21	27	31
Anencephaly	32	51	72	88	66	77	36	44	35	50	28	39
Spina bifida-cephalic	7	14	24	38	24	36	11	18	2	6	1	6
Spina bifida-caudal	34	47	49	73	24	34	38	50	31	56	19	36
Spina bifida-total	44	69	76	117	52	74	51	71	37	67	21	45
Hydrocephaly	28	53	34	68	29	53	30	56	76	130	35	70
Cephalocele	10	17	10	23	12	15	18	23	14	24	7	20
Microcephaly	11	39	6	16	13	29	15	30	54	98	25	55
An/microtia	24	43	29	53	24	41	33	42	72	92	33	68
CHD-conotruncal	14	20	26	37	28	48	38	53	99	142	40	57
CHD-septal	41	72	33	65	80	143	135	219	313	528	198	303
CHD-valvular	2	3	6	9	9	12	8	11	18	24	12	16
CHD-other severe	9	14	17	24	16	24	22	24	37	50	11	24
CHD-unspecified	87	113	64	97	30	42	13	18	14	36	16	33

CHD-severe-total (I-I)	11	17	20	31	18	45	30	61	101	176	26	53
CHD-severe-conotruncal (I-I)	9	14	19	25	14	26	22	33	76	108	17	32
CHD-severe-non-conotruncal (I-I)	2	3	1	6	4	19	8	28	25	68	9	21
Cleft palate only	22	43	16	41	22	41	11	28	50	84	35	70
Cleft lip ± cleft palate	87	108	94	118	62	81	62	83	101	155	76	127
Cleft lip only	25	27	36	39	17	19	14	19	23	30	22	26
Cleft lip and palate	62	81	58	79	45	62	48	64	78	125	54	101
Esophageal atresia	12	21	20	45	11	22	9	15	12	24	7	19
Duodenal atresia	7	11	5	13	5	10	3	13	6	30	7	29
Anal atresia	18	39	23	54	21	45	13	37	32	90	24	62
Ambiguous genitalia	5	23	4	20	4	19	0	8	7	27	5	18
Hypospadias-total	61	72	51	66	52	70	54	57	107	126	68	93
Hypospadias-distal	53	62	43	55	43	53	52	55	97	109	62	80
Hypospadias-proximal	6	7	7	9	4	10	1	1	8	13	4	7
Absent kidney/s	4	18	1	19	6	28	4	12	9	30	10	27
Polycystic kidneys	13	30	15	40	16	31	21	35	38	64	19	41
Hydronephrosis	10	25	11	28	34	50	65	78	167	220	108	166
Talipes equinovarus	96	149	128	192	85	111	69	94	132	170	76	127
Talipes calcaneovalgus	60	81	62	90	53	63	62	78	73	100	35	59
Polydactyly-post-axial	61	84	80	100	78	96	65	86	149	190	90	121
Polydactyly-pre-axial	22	24	17	24	23	26	19	31	52	70	32	37
Polydactyly-others	3	5	3	6	4	8	1	3	7	12	5	8
Syndactyly-toes 2–3	21	27	22	25	24	30	9	20	12	19	10	19
Syndactyly-other types	16	40	21	48	16	33	8	23	43	79	20	40
LRD-TT: amputation	17	21	15	26	13	20	9	17	22	43	5	13
LRD-TTH: hypoplasia	4	11	4	9	1	2	0	3	2	9	3	4
LRD-pre-axial	2	4	1	11	3	12	5	8	5	19	2	12
Hip-subluxation	95	121	65	79	45	49	53	58	69	84	35	49
Hip-dislocation	0	3	1	6	1	4	5	6	2	3	1	5
Arthrogryposis	3	9	4	19	4	17	6	15	12	29	2	17
Diaphragmatic hernia	17	30	23	48	16	35	27	36	40	55	29	42
Abdominal muscle deficiency	0	13	0	10	1	7	0	3	3	6	2	7
Pectoralis hypoplasia	2	4	2	2	1	5	2	6	3	6	2	4
Skin ring constriction	3	3	0	6	2	5	0	4	1	11	0	4
Down total	148	148	183	183	188	188	161	161	358	358	260	260
Down ≤19 YMA	13	13	13	13	14	14	17	17	18	18	20	20
Down ≥35 YMA	63	63	85	85	90	90	83	83	228	228	167	167

Spina bifida-cephalic, cervical, thoracic; spina bifida-caudal, lumbal, sacral; CHD, congenital heart disease; (I-I), CHD grouped as Ionescu-Ittu et al. [2009]; hypospadias-distal, balanic, balano-prepucial; hypospadias-proximal, penile, scrotal, perineal; LRD, limb reduction defect; TT, transverse terminal; TTH, transverse terminal hypoplasia (includes brachydactyly); YMA, years of maternal age; Down syndrome always considered as isolated by definition.

[a]Births.

APPENDIX B. Number of cases by specific congenital anomaly types in their isolated (Iso) and total (isolated plus associated) forms, by year of birth, in Argentina.

	Period (years)											
	1982–1987 (349,895[a])		1988–1992 (360,113[a])		1993–1997 (350,069[a])		1998–2001 (241,902[a])		2002–2004 (193,509[a])		2005–2007 (147,853[a])	
	Iso	Total	Iso	Total	Iso	Total	Iso	Total	Iso	Total	Iso	Total
Omphalocele	31	79	33	81	33	76	24	63	34	67	21	55
Gastroschisis	12	18	21	28	49	60	62	73	76	82	85	110
Anencephaly	180	234	223	288	224	271	160	197	134	167	43	54
Spina bifida-cephalic	22	52	34	63	62	98	52	84	46	72	3	7
Spina bifida-caudal	98	157	125	192	129	206	109	168	110	170	45	88
Spina bifida-total	132	242	160	263	194	310	163	255	158	246	49	98
Hydrocephaly	83	163	133	243	185	358	137	258	105	214	77	171
Cephalocele	37	63	42	67	42	71	46	74	41	61	15	29
Microcephaly	36	88	51	107	40	108	22	74	19	51	17	44
An/microtia	64	145	95	179	106	170	51	99	53	94	27	60
CHD-conotruncal	49	78	78	118	125	185	103	146	49	119	78	59
CHD-septal	145	214	144	241	361	558	250	437	145	424	144	223
CHD-valvular	13	16	18	22	25	33	15	27	13	30	18	18
CHD-other severe	17	24	34	50	68	86	59	81	17	63	34	36
CHD-unspecified	46	140	71	153	61	144	43	59	46	12	71	15
CHD-severe-total (I-I)	38	70	49	97	96	158	89	134	69	108	31	58
CHD-severe-conotruncal (I-I)	35	53	44	70	81	108	67	89	43	56	21	40
CHD-severe-non-conotruncal (I-I)	3	17	5	27	15	50	22	45	26	52	10	18
Cleft palate only	41	140	46	153	47	144	50	59	27	12	23	15
Cleft lip ± cleft palate	300	368	290	374	319	404	254	323	183	266	99	160
Cleft lip only	97	111	87	103	96	111	76	85	46	57	29	38
Cleft lip and palate	203	257	203	271	223	293	178	238	137	209	70	122
Esophageal atresia	50	91	48	95	40	82	33	78	39	65	21	49
Duodenal atresia	7	36	18	33	22	38	12	36	7	20	4	17
Anal atresia	47	133	67	177	82	192	40	135	45	121	26	83
Ambiguous genitalia	13	67	9	58	13	50	10	40	9	34	7	29
Hypospadias-total	156	204	183	225	145	186	122	149	99	124	55	70
Hypospadias-distal	133	170	165	198	121	148	108	121	86	102	51	61
Hypospadias-proximal	15	21	11	16	20	30	9	20	7	15	4	6
Absent kidney/s	1	15	2	26	6	61	14	62	16	56	17	49
Polycystic kidneys	9	31	13	39	41	79	53	87	49	83	27	47
Hydronephrosis	9	29	21	47	57	117	60	100	60	110	45	115
Talipes equinovarus	298	481	316	511	355	519	170	284	153	303	90	211
Talipes calcaneovalgus	106	176	88	147	90	138	52	86	42	71	23	42
Polydactyly-post-axial	268	341	309	384	278	367	215	283	188	237	97	143
Polydactyly-pre-axial	61	81	76	105	91	115	69	96	61	71	36	58
Polydactyly-others	8	19	8	26	11	26	3	7	10	14	3	6
Syndactyly-toes 2–3	41	61	37	53	31	53	23	37	6	12	11	17
Syndactyly-other types	41	136	45	145	52	135	23	95	19	63	18	56
LRD-TT: amputation	53	92	43	93	34	80	31	73	40	81	16	30

LRD-TTH: hypoplasia	13	54	11	42	10	33	8	26	7	19	5	11
LRD-pre-axial	9	56	10	45	18	57	5	46	5	30	3	18
Hip-subluxation	797	904	458	530	319	371	94	125	56	69	13	29
Hip-dislocation	20	81	23	82	10	40	3	8	6	14	1	13
Arthrogryposis	10	33	21	53	27	109	19	82	14	64	0	13
Diaphragmatic hernia	33	59	38	66	71	103	78	105	50	81	47	73
Abdominal muscle deficiency	4	23	4	28	3	21	2	19	1	17	0	9
Pectoralis hypoplasia	9	32	20	40	18	48	5	19	4	16	3	13
Skin ring constriction	2	20	4	30	3	35	4	26	1	27	0	5
Down total	604	604	654	654	643	643	512	512	369	369	273	273
Down $\leq$19 YMA	51	51	48	48	55	55	45	45	20	20	32	32
Down $\geq$35 YMA	331	331	350	350	339	339	250	250	193	193	135	135

Spina bifida-cephalic, cervical, thoracic; spina bifida-caudal, lumbal, sacral; CHD, congenital heart disease; (I-I), CHD grouped as Ionescu-Ittu et al. [2009]; hypospadias-distal, balanic, balano-prepucial; hypospadias-proximal, penile, scrotal, perineal; LRD, limb reduction defect; TT, transverse terminal; TTH, transverse terminal hypoplasia (includes brachydactyly); YMA, years of maternal age; Down syndrome always considered as isolated by definition.

[a]Births.

APPENDIX C. Number of cases by specific congenital anomaly types in their isolated (Iso) and total (isolated plus associated) forms, by year of birth, in Brazil.

	Period (month/years)											
	1982–1987 (291,825[a])		1988–1992 (242,380[a])		1993–1997 (189,106[a])		1998–2002 (188,609[a])		2003–2006/2005 (102,751[a])		2007/2005–2007 (92,843[a])	
	Iso	Total	Iso	Total	Iso	Total	Iso	Total	Iso	Total	Iso	Total
Omphalocele	18	60	39	76	30	89	35	117	20	61	23	58
Gastroschisis	11	17	17	23	32	56	90	101	62	69	68	82
Anencephaly	126	169	98	129	110	157	156	208	92	115	42	64
Spina bifida-cephalic	11	28	13	31	15	34	29	61	19	34	6	13
Spina bifida-caudal	64	126	68	115	92	161	123	216	64	107	52	114
Spina bifida-total	80	175	86	157	111	203	160	288	88	149	64	132
Hydrocephaly	76	128	106	202	197	358	220	394	128	226	78	181
Cephalocele	24	44	31	64	38	65	41	72	32	59	11	30
Microcephaly	33	91	27	61	28	72	27	86	31	55	19	40
An/microtia	17	58	28	62	24	75	14	51	17	41	16	38
CHD-conotruncal	30	48	58	88	76	126	84	131	48	66	36	63
CHD-septal	127	190	183	279	136	263	219	431	125	234	93	175
CHD-valvular	7	10	22	33	21	31	23	34	10	13	9	16
CHD-other severe	15	25	22	37	30	53	34	54	12	21	17	30
CHD-unspecified	53	122	49	99	23	60	64	95	30	54	16	30
CHD-severe-total (I-I)	28	46	53	91	55	105	58	138	29	57	27	43
CHD-severe-conotruncal (I-I)	22	35	44	66	47	71	46	79	20	37	19	37
CHD-severe-non-conotruncal (I-I)	6	11	9	25	8	34	12	59	9	20	8	6
Cleft palate only	35	93	32	91	19	70	33	102	21	61	7	39
Cleft lip ± cleft palate	178	233	157	222	148	207	147	236	72	114	61	121
Cleft lip only	63	77	55	69	59	73	57	70	27	36	24	35
Cleft lip and palate	115	156	102	153	89	134	90	166	45	78	37	86
Esophageal atresia	39	79	33	63	22	60	28	84	19	46	6	21
Duodenal atresia	7	13	14	28	13	32	9	27	9	20	5	19
Anal atresia	30	91	28	103	16	89	15	85	9	50	4	29
Ambiguous genitalia	21	67	12	54	11	58	18	76	16	38	4	47
Hypospadias-total	349	414	297	354	250	308	323	385	193	224	143	188
Hypospadias-distal	294	343	251	292	219	263	286	337	178	198	127	155
Hypospadias-proximal	43	56	34	44	18	27	31	38	13	20	9	20
Absent kidney/s	3	29	18	56	19	72	33	83	18	42	9	36
Polycystic kidneys	13	43	30	71	46	113	96	155	32	58	24	52
Hydronephrosis	26	55	58	105	133	213	287	388	98	138	128	190
Talipes equinovarus	395	556	390	569	279	451	320	500	226	344	148	295
Talipes calcaneovalgus	113	177	415	482	129	170	146	200	111	141	72	111
Polydactyly-post-axial	685	791	626	699	482	565	467	574	311	382	282	356
Polydactyly-pre-axial	64	79	40	63	39	55	36	56	26	36	25	44
Polydactyly-others	13	24	9	20	9	19	9	23	2	10	4	8
Syndactyly-toes 2–3	73	103	51	70	40	53	44	65	16	23	15	27
Syndactyly-other types	59	134	38	107	24	77	21	72	16	43	7	42
LRD-TT: amputation	59	85	41	76	38	77	34	74	34	61	6	18

LRD-TTH: hypoplasia	10	32	14	31	8	17	6	13	5	11	2	5
LRD-pre-axial	3	22	6	18	7	28	2	30	6	19	2	10
Hip-sub-dislocation	572	661	523	583	309	356	376	432	168	193	119	150
Hip-dislocation	42	65	14	36	12	34	3	11	0	4	1	6
Arthrogryposis	4	14	9	27	13	73	29	118	8	40	2	14
Diaphragmatic hernia	12	33	19	59	34	72	53	94	34	51	19	34
Abdominal muscle deficiency	2	20	0	21	1	27	1	37	0	17	0	8
Pectoralis hypoplasia	3	7	6	8	1	2	2	3	0	2	0	3
Skin ring constriction	0	6	0	17	2	26	1	22	2	15	2	13
Down total	342	342	333	333	277	277	312	312	204	204	147	147
Down $\leq$19 YMA	20	20	23	23	29	29	19	19	19	19	12	12
Down $\geq$35 YMA	158	158	145	145	125	125	157	157	102	102	76	76

Spina bifida-cephalic, cervical, thoracic; spina bifida-caudal, lumbal, sacral; CHD, congenital heart disease; (I-I), CHD grouped as Ionescu-Ittu et al. [2009]; hypospadias-distal, balanic, balano-prepucial; hypospadias-proximal: penile, scrotal, perineal; LRD, limb reduction defect; TT, transverse terminal; TTH, transverse terminal hypoplasia (includes brachydactyly); YMA, years of maternal age; Down syndrome always considered as isolated by definition.

[a]Births.