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Review article

Concentrations of blood folate in Brazilian studies prior to and after fortification of wheat and cornmeal (maize flour) with folic acid: a review

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ABSTRACT

Background: In July 2004, the Brazilian Ministry of Health through the National Health Surveillance Agency made the fortification of wheat flour and cornmeal (maize flour) with iron and folic acid mandatory, with the intention of reducing the rate of diseases such as neural tube defects.
Objective: The aim of the study was to investigate the impact of the folic acid fortified wheat flour and cornmeal on serum and red blood cell folate levels and on the reduction of neural tube defects in different Brazilian studies.
Methods: In order to compare folate concentrations in the Brazilian population prior to and following the implementation of mandatory fortification of wheat and cornmeal, studies that involved blood draws between January 1997 and May 2004 (pre-fortification period), and from June 2004 to the present (post-fortification period) were chosen. The data search included PubMed and Scopus databases as well as the Brazilian Digital Library of Theses and Dissertations. The following keywords were employed for the query: folate, folic acid, fortification, Brazil, healthy population, the elderly, children and pregnant women.
Results: A total of 47 Brazilian studies were selected; 26 from the pre-fortification period and 22 after the fortification implementation. The studies were classified according to the cohort investigated (pregnant women, children, adolescents, adults and the elderly). After the implementation of flour fortification with folic acid in Brazil, serum folate concentrations increased in healthy populations (57% in children and adolescents and 174% in adults), and the incidence of neural tube defects dropped.
Conclusion: Folic acid fortification of wheat flour and cornmeal increased the blood folate concentrations and reduced the incidence of neural tube defects.

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Introduction

Folic acid (FA) is a hydrosoluble vitamin essential for human health; its main roles in cell metabolism involve DNA synthesis and supplying methyl groups for homocysteine (Hcy), DNA, protein and lipid methylation reactions.[1]

The term folate is used to designate the polyglutamate form of water-soluble B vitamin present in edibles, while the term folic acid corresponds to the monoglutamate form used in supplements and in the fortification of food.[2] Folate rich foods include: green vegetables (broccolis, lettuce, spinach and asparagus), beans, fruit (lemons, bananas and melons), dry cereals, whole-grains, liver, kidney and mushrooms.[3] The physiological folate requirements increase when there is a corresponding increase in cell division such as during pregnancy, lactation and in early childhood; or whenever individuals are afflicted with certain diseases, such as hemolytic anemia, leukemia and other malignant diseases, as well as in alcoholism.[4]

It is believed to be difficult to obtain the required intake of this vitamin by means of a balanced diet alone (without fortified foods) when there is an increase in physiological necessities. A normal diet supplies around 0.25 mg of folate/day, considering a diet of 2200 calories per day. The difficulty in fulfilling the requirements may be explained by the low bioavailability of folate in foods and the low dietary intake of foods that are natural sources of this vitamin. Furthermore, high temperature processing of foods results in considerable loss of folate, reducing its content by 50%.[5]

The recommended dietary allowance (RDA), estimated average requirement (EAR) and the tolerable upper intake level (UL) reference values for folate differ according to age (children, adolescents and adults) remembering that intake requirements are higher for pregnant (RDA 600 μg/day and EAR 520 μg/day) and breast-feeding women (RDA 500 μg/day and EAR 450 μg/day).[6] During pregnancy, cells multiply intensively due to the widening of the uterus, placental development, increase in blood volume and fetal development, which increases folate and B12 vitamin necessities accordingly.[7]

Adequate intake of these vitamins is essential, since folate insufficiency has been identified as a risk factor for congenital disorders especially neural tube defects (NTDs). They result from neural tube closing failure during the early development of the embryo, typically between the 21st and 28th day after conception, most frequently resulting in anencephaly and spina bifida.

Since pregnancy is not always planned, it is important that women of child-bearing age have access to a suitable quantity of FA, at least one month prior to becoming pregnant. Accordingly, it is recommended that women of child-bearing age consume 400 μg of FA daily, via fortified foods, supplements or both, in addition to the quantity they acquire from their normal daily diet.[6] Considering the difficulties to obtain the folate requirements from a normal balanced diet, several countries decided to implement mandatory FA fortification of foods, starting with the United States in 1998, followed shortly by Canada, Chile and several others.

In Brazil, the Ministry of Health through the National Health Surveillance Agency (ANVISA) made the iron and FA fortification of wheat and cornmeal mandatory in July 2004, with the intention of reducing the rate of pathologies, like NTDs, nationally. When the RDC Resolution no. 344 was issued on December 13, 2002, ANVISA dictated that all wheat flour and cornmeal, whether sold directly to consumers or to the food industry for the manufacture of edibles, must be enriched with iron and FA. It was established that every 100 g of wheat flour and cornmeal must contain at least 4.2 mg of iron and 150 μg of FA.[8] However, no nationwide studies have been carried out to evaluate the concentrations of folate consumed by the Brazilian population prior to and following the mandatory implementation of fortified wheat flour and cornmeal. Accordingly, the purpose of this review is to investigate the impact of the FA fortification of wheat flour and cornmeal on serum and red blood cell folate levels, and to evaluate the reduction of NTDs in different strata of the Brazilian population.

Methods

In order to compare folate concentrations in the Brazilian population prior to and following the implementation of mandatory fortification of wheat flour and cornmeal, studies that involved blood draws between January 1997 and May 2004 (the pre-fortification period), and from June 2004 to the present (the post-fortification period) were chosen. Data reviewed included PubMed and Scopus databases as well as the Brazilian Digital Library of Theses and Dissertations. The following keywords were employed in the query: folate, folic acid, fortification, Brazil, healthy population, the elderly, children and pregnant women.

Studies in which the sample collection included both time periods were classified as "pre-fortification studies", as long as the sample collection period prior to June 2004 was longer than the period after June 2004. Likewise, studies in which the collection period after June 2004 was greater than the period prior to mandatory fortification were classified as "post-fortification studies". A number of studies did not specify the sample collection period; in these cases, emails were sent to the respective corresponding authors in order to determine this information.

Transversal and/or prospective studies were selected, without interventions, carried out on different cohorts of the Brazilian population, such as pregnant women, neonates, children and adolescents, adults and the elderly. The studies that evaluated the concentrations of folate in unhealthy populations were also selected and the data are presented in the Tables below but were not taken into consideration in the whole evaluation between the pre- and post-fortification periods. For consistency purposes , studies that presented folate concentrations expressed in ng/mL had their values converted into nmol/L using a conversion factor of 2.266[9] for this review.

In order to evaluate the concentrations of serum folate between the pre- and post-fortification periods, the increase of serum concentrations was estimated in children and adolescents and adults. Pregnant women were not considered for this evaluation, because the studies found presented great variations in the gestational age of the subjects within this cohort. Neonates and the elderly were not evaluated either, because few studies involving these cohorts were found for the two periods considered.

Results

A total of 47 Brazilian studies were selected, including 26 from the pre-fortification and 22 from the post-fortification periods. The studies were classified according to the cohort investigated (pregnant women, children, adolescents, adults and the elderly). Several articles analyzed more than one type of population in the same study and so that these studies may appear in more than one Table in the results section.

Tables 1 to 7 present the characteristics of the selected studies, including where they were carried out, the period of the sample collection, the characteristics of the evaluated cohort, the number of individuals involved in the study (n) and the method used for quantifying the folate.

Tables 1 to 3 present the characteristics of the studies that evaluated the concentrations of serum folate on different healthy populations, while Tables 4 and 5 present the

Table 1 - Serum folate concentrations in healthy pregnant women and neonates.

Pre-fortification							Post-fortification						
References	Blood collection period	Place	Population characteristics)	n	Serum folate concentration (nmol/L)	Methods	References	Blood collection period	Place	Population characteristics	n	Serum folate concentration (nmol/L)	Methods
Pregnant women							*Pregnant women*						
Thame. Guerra-Shinohara et al. 2002[10]	February to October 1997	São Paulo - SP	Pregnant women with NTD babies (30.6 ± 5.5 weeks)	38	19.6 (± 8.7)[a]	Ionic capture (IMx System®. ABBOTT)	Guerra-Shinohara. Pereira et al. 2010[13]	February 2004 to December 2005	São Paulo - SP	Pregnant women (12 weeks)	88	27.4 (19.6; 39.3)[d]	CL (Immulite®. DPC Med Lab)
Guerra-Shinohara. Paiva et al. 2002[11]	August to November 1999	Jundiaí - SP	Women in labor (38 to 42 weeks)	69	13.9 (± 5.6)[a]	Ionic capture (IMx System®. ABBOTT)	Kubota. 2010[14]	February 2004 to December 2005	São Paulo - SP	Pregnant women (8 to 12 weeks)	38	24.4 (± 13.6)[a] 23.8 (13.5 – 28.4)[d]	CL (Immulite®. DPC Med Lab)
Guerra-Shinohara. Morita et al. 2004[7]	2001	Sorocaba - SP	Women in labor (38 to 42 weeks)	112	12.9 (12.0 - 14.0)[b]	Ionic capture (IMx System®. ABBOTT)				16 weeks	50	24.3 (± 17.5)[a] 22.1 (15.7 – 29.2)[d]	
										28 weeks	50	22.3 (± 9.9)[a] 20.9 (15.0 – 29.7)[d]	
										36 weeks	50	21.4 (± 7.7)[a] 20.6 (15.5 – 25.6)[d]	
Barbosa. Stabler. Machado et al. 2008[12]	April 2001 to May 2003	Sorocaba - SP	Women in labor (38 to 42 weeks)	275	12.5[c] 13.7 (± 6.8)[a]	Ionic capture (IMx System®) and CL (Immulite®)							
Neonates							*Neonates*						
Guerra-Shinohara. Paiva et al. 2002[11]	August to November 1999	Jundiaí - SP	Blood sample from umbilical cord	69	27.9 (± 3.9)[a]	Ionic capture (IMx System®. ABBOTT)							
Guerra-Shinohara. Morita et al. 2004[7]	2001	Sorocaba - SP	Blood sample from placental neonatal vein	112	30.9 (29.8 - 32.1)[b]	Ionic capture (IMx System®. ABBOTT)							
Couto. Moreira et al. 2007[15]	February to December 2000	Salvador - BA	Neonates	143	17.7 (± 8.0)[a]	ECL immunoassay (ECLIA, Roche)							

CL: chemiluminescence; ECL: electrochemiluminescence; NTD: neural tube defects.
[a] Serum folate concentration: mean (± SD).
[b] Serum folate concentration: geometric means (95% CI).
[c] Serum folate concentration: median.
[d] Serum folate concentration: median (P25-P75).

Table 2 - Serum folate concentrations in healthy pregnant women and neonates.

Pre-fortification							Post-fortification						
References	Blood collection period	Place	Population characte-ristics	n	Serum folate concen-tration (nmol/L)	Methods	References	Blood collection period	Place	Population characte-ristics	n	Serum folate concen-tration (nmol/L)	Methods
Children and adolescents							*Children and adolescents*						
Félix, Leistner et al., 2004[16]	2000 to 2001	Porto Alegre - SP	Children without NTD	44	12.8 (± 9.4)[a]	Ionic capture (IMx System®. ABBOTT)	Hadler, Sigulem et al., 2008[20]	2005	Goiânia - GO	Children (6 to 24 months)	157	29.5 (± 12.2)[a]	CL (Bayer)
Do Prado, D'almeida et al., 2006[17]	November 2002 to September 2003	São Paulo - SP	Healthy children and adolescents (29 girls)	32	14.3 (± 5.4)[a]	Ionic capture (IMx System®. ABBOTT)	Steluti, Martini et al., 2011[21]	2006	Indaiatuba - SP	Adolescents: - Girls - Boys	 36 53	 21.5 (± 9.3)[a] 20.4 (± 6.6)[a]	ECL (Elecsys®)
Galdieri, Arrieta et al., 2007[18]	2002 to 2004	São Paulo - SP	Healthy children	30	18.8 (± 6.8)[a]	HPLC	Cardoso, Scopel et al., 2012[22]	December 2007	Acrelândia - AC	Children (6 months to 10 years old)	1032	23.3 (17.7; 30.3)[d]	FIA
Gonçalves, D'almeida et al., 2007[19]	November 2002 to September 2003	São Paulo - SP	Healthy girls (children and adolescents)	52	15.1 (± 5.1)[a]	Ionic capture (IMx System®. ABBOTT)	Fialho, 2012[23]	2008	Fortaleza - CE	Children and adolescents H. pylori (-)	47	30.4 (± 8.4)[a]	CL (Immulite®, DPC Med Lab)
							Bigio 2011[24]	2008 to 2010	São Paulo - SP	Adolescents: - Girls - Boys	 82 101	 19.3 (± 6.6)[a] 18.6 (± 6.6)[a]	ECL (Elecsys®)
							Da Costa, Schtscherbyna et al., 2013[25]	2005 to 2006	Rio de Janeiro - RJ	Girls - 11 to 14 years old - 15 to 19 years old	 25 18	 24.7[c] 27.9[c]	RIA (Dualcount, DPC® Medlab)
Elderly							*Elderly*						
Moriguti, Ferriolli et al., 1999[26]	1998	Ribeirão Preto - SP	Elderly (men)	8	15.9 (± 2.5)[a]	?	Tassino, Campos et al., 2009[27]	2006 to 2007	Natal - RN	Low income elderly	205	26.3 (± 10.2)[a]	CL (Immulite®, DPC Med Lab)
							Xavier, Costa et al., 2010[28]	November 2006 to September 2007	Campinas - SP	Elderly	250	24.7 (± 6.9)[a]	ECL (Elecsys®)
							Coussirat, 2010[29]	July 2005 to June 2010	Porto Alegre - RS	Elderly	420	28.6 (± 11.3)[a]	CL (VITROS ECi Immuno-diagnostic System -J&J)

NTD: neural tube defects; CL: chemiluminescence; ECL: electrochemiluminescence; HPLC: high-performance liquid chromatography; FIA: fluoroimmunoassay.
[a] Serum folate concentration: mean (± SD).
[b] Serum folate concentration: geometric means (95% CI).
[c] Serum folate concentration: median.
[d] Serum folate concentration: median (P25-P75).
Missing information was represented with a question mark (?).

characteristics of the studies involving unhealthy populations. Tables 6 and 7 present the characteristics of the studies that evaluate the total blood or red blood cell folate concentrations among healthy and unhealthy populations, respectively.

Increases of 57% and 174% of the serum folate concentration were observed between the pre- and post-fortification periods for the children and adolescents cohort and for the healthy adults cohort, respectively.

Of the total number of studies encountered, 32 (68%) were held at southeastern geographical region of Brazil, while 6 (13%), 1 (2%), 2 (4%) and 6 (13%) studies were conducted in the southern, mid-west, northern and northeastern geographical regions, respectively.

Discussion

The need to reduce the incidence of congenital disorders in the population has led some countries to adopt a program to fortify foods with FA. Other countries, especially in Europe, have implemented special women's healthcare initiatives,

Table 3 - Serum folate concentrations in healthy adults.

Pre-fortification							Post-fortification						
References	Blood collection period	Place	Population characteristics	n	Serum folate concentration (nmol/L)	Methods	References	Blood collection period	Place	Population characteristics	n	Serum folate concentration (nmol/L)	Methods
Adults							*Adults*						
Martins, D'Almeida et al., 2003[30]	?	São Paulo - SP	Day time working men Shift working men	22 30	19.2 (± 8.9)[a] 11.5 (± 5.1)[a]	CL (ACS :180®)	Mendes, Biselli et al., 2010[40]	February 2005 to February 2008	São José do Rio Preto - SP	Mothers of healthy children, polymorphism DHFR ins/ins 19bp intron 1 ins/ins ins/del del/del	42 85 57	35.3[c] 32.0[c] 33.1[c]	CL (Immulite®, DPC Med Lab)
Félix, Leistner et al., 2004[16]	2000 to 2001	Porto Alegre - RS	Mothers of healthy children	44	8.8 (± 4.0)[a]	Ionic capture (IMx System®. ABBOTT)	Xavier, Costa et al., 2010[28]	November 2006 to September 2007	Campinas - SP	Adults	250	23.8 (± 9.2)[a]	ECL (Elecsys®)
Pereira, Schettert et al., 2004[31]	2000	São Paulo - SP	Adults	209	12.1 (± 4.3)[a]	Ionic capture (IMx System®. ABBOTT)	Barnabé, 2010[41]	?	Campinas - SP	Adults	28	23.3 (± 6.9)[a]	ECL (Elecsys®)
Tavares, Vieira-Filho et al., 2004[32]	July 1998	Marabá – PA	Parkatêjê Índians Women Men	34 56	9.3 (± 2.9)[a] 7.0 (± 2.7)[a]	CL (Immulite®, DPC Med Lab)	Minozzo, Deimling et al., 2010[42]	July 2005 to July 2006	Porto Alegre - RS	Healthy men	53	14.4 (± 5.7)[a]	CL (Access Immunoassay System Beckman Instruments)
Helfenstein, Fonseca et al., 2005[33]	2003 to 2004	São Paulo - SP	Healthy adults	56	13.6 (± 0.9)[e]	AxSYM Analyzer	Braga, Vannucchi et al., 2011[43]	August 2008 to November 2009	Ribeirão Preto - SP	Healthy adults	9	32.6 (± 11.8)[a]	CL (Immulite®, DPC Med Lab)
Muniz, Siqueira et al., 2006[34]	1999 to 2001	Recife - PE	Healthy adults	108	17.5 (± 7.0)[a]	CL (ACS :180®)	Vinha, Jordão et al., 2011[44]	2007 to 2008	Ribeirão Preto - SP	Adults undergoing surgery of burn sequelae[1]	8	17.0[c]	CL (Immulite®, DPC Med Lab)
Faria-Neto, Chagas et al., 2006[35]	1999 to 2000	São Paulo - SP	Adults with normal or almost normal arteries	88	17.4 (± 8.2)[a]	RIA (Dualcount, DPC® Medlab)	Chiarani, 2012[45]	?	Porto Alegre - RS	Healthy adults	30	23.5 (± 2.2)[a]	CL
Galdieri, Arrieta et al., 2007[18]	2002 to 2004	São Paulo - SP	Mothers of healthy children	25	13.0 (3.1)[a]	HPLC	Giusti, 2012[46]	November 2008 to September 2011	São Paulo - SP	Women with no history of miscarriage	264	31.8 (± 1.5)[a]	Microbiological assay
Almeida, Tomita et al., 2008[36]	March 2003 to May 2005	São Paulo - SP	Low income women	1085	14.3 (10.2 – 20.9)[d]	Immunoassay (PerkinElmer®)	De Carvalho, Muniz et al., 2013[47]	2005 to 2008	Recife - PE	Healthy adults	51	34.3 (± 6.8)[a]	ECL (Elecsys®)
Barbosa, Stabler, Trentin et al., 2008[37]	2003	Sorocaba - SP	Healthy women	102	15.2 (14.1-16.4)[b] 16.3 (± 6.0)[a]	CL (Immulite®, DPC Med Lab)							
Biselli, Guerzoni et al., 2010[38]	2001 and 2004	São José do Rio Preto - SP	Adults polymorphism MTHFR A1298C AA AC CC	54 49 5	10.9 (± 5.0)[a] 10.7 (± 6.6)[a] 19.0 (± 4.5)[a]	CL (Immulite®, DPC Med Lab)							
Blume, Boni et al., 2012[39]	2000 to 2005	Porto Alegre - RS	Obese adults (136 women and 34 men)	170	18.4 (11.1 – 26.3)[d]	CL (Centaur®)							

NTD: neural tube defects; MTHFR: methylenetetrahydrofolate reductase; DHFR: dihydrofolate reductase; CL: chemiluminescence; ECL: electrochemiluminescence.2
[a] Serum folate concentration: mean (± SD).
[b] Serum folate concentration: geometric means (95% CI).
[c] Serum folate concentration: median.
[d] Serum folate concentration: median (P25-P75).
[e] Serum folate concentration: mean (± SEM).
The burns occurred at least one year before the study. Missing information is represented with a question mark (?).

Table 4 - Serum folate concentrations in unhealthy populations.

Pre-fortification

References	Blood collection period	Place	Population characteristics	n	Serum folate concentration (nmol/L)	Methods
NTD and abortion						
Thame, Guerra-Shinohara et al., 2002[10]	February to October 1997	São Paulo - SP	Pregnant women carrying fetuses with NTD	17	12.6 (± 4.4)[a] 11.3[c]	Ionic capture (IMx System®. ABBOTT)
Félix, Leistner et al., 2004[16]	2000 to 2001	Porto Alegre - RS	Mothers of children with NTD Children with NTD	41 41	16.7 (± 10.2)[a] 25.8 (± 15.2)[a]	Ionic capture (IMx System®. ABBOTT)
Cunha, Hirata et al., 2002[48]	?	São Paulo - SP	Children with NTD, polymorphism MTHFR C677T CC CT/TT	 12 13	 24.0 (± 8.0)[a] 30.0 (± 7.0)[a]	Ionic capture (IMx System®. ABBOTT)
Cardiometabolic alterations						
Helfenstein, Fonseca et al., 2005[33]	2003 to 2004	São Paulo - SP	Adults with: - DM2 and MI - DM2 - MI	 43 50 47	 20.8 (± 1.6)[e] 21.1 (± 1.6)[e] 14.0 (± 0.9)[e]	AxSYM Analyzer, ABBOTT
Muniz, Siqueira et al., 2006[34]	1999 to 2001	Recife - PE	Adults with CAD	93	14.4 (± 6.6)[a]	CL (ACS :180®)
Galdieri, Arrieta et al., 2007[18]	2002 to 2004	São Paulo	Children with congenital heart defects Mothers of children with congenital heart defects	47 44	26.8 (± 24.3)[a] 20.0 (± 10.7)[a]	HPLC
Faria-Neto, Chagas et al., 2006[35]	1999 to 2000	São Paulo - SP	Adults with CAD	148	16.8 (± 7.5)[a]	RIA (Dualcount, DPC® Medlab)
Melo, Persuhn et al., 2006[49]	2003	Balneário Camboriu - SC	Adults with DM2, polymorphism MTHFR G1793A GG GA/AA	 78 5	 15.8[c] 8.0[c]	RIA (Dualcount, DPC® Medlab)
Uehara e Rosa, 2008[50]	2002 to 2003	Rio de Janeiro - RJ	Adults with MS: - Men - Women	 24 39	 13.4 (± 7.9)[e] 14.5 (± 8.2)[e]	RIA (Dualcount, DPC® Medlab)
Scorsatto, Uehara et al., 2011[51]	2002 to 2003	Rio de Janeiro - RJ	Women with MS	38	15.7 (± 10.7)[a]	CL (Immulite®, DPC Med Lab)
Biselli, Guerzoni et al., 2010[38]	2001 to 2004	São José do Rio Preto - SP	Adults with CAD polymorphism MTHFR A1298C AA AC CC	 101 67 7	 11.1 (± 6.8)[a] 13.1 (± 6.6)[a] 12.0 (± 1.8)[a]	CL (Immulite®, DPC Med Lab)

Post-fortification

References	Blood collection period	Place	Population characteristics	n	Serum folate concentration (nmol/L)	Methods
NTD and abortion						
Guerra-Shinohara, Pereira et al., 2010[13]	February 2004 to December 2005	São Paulo - SP	Women who had spontaneous abortion	12	26.3 (20.9; 40.7)[d]	CL (Immulite®, DPC Med Lab)
Giusti, 2012[46]	November 2008 to September 2011	São Paulo - SP	Women with: Primary abortion Secondary abortion	 117 139	 32.3 (31.1 – 33.4)[a] 34.2 (33.2 – 35.2)[a]	Microbiological assay
Cardiometabolic alterations						
Scorsatto, Uehara et al., 2011[51]	2008 to 2009	Rio de Janeiro - RJ	Women with MS	55	12.1 (± 3.8)[a]	CL (Immulite®, DPC Med Lab)

NTD: neural tube defects; DM2: type 2 diabetes mellitus; MI: myocardial infarction; MS: metabolic syndrome; CAD: coronary artery disease; MTHFR: methylenetetrahydrofolate reductase; CL: chemiluminescence; HPLC: high-performance liquid chromatography; RIA: radioisotope assay.
[a] Serum folate concentration: mean (± SD).
[b] Serum folate concentration: geometric means (95% CI).
[c] Serum folate concentration: median.
[d] Serum folate concentration: median (P25-P75).
[e] Serum folate concentration: mean (± SEM).
The missing information was represented with question mark (?).

Table 5 - Serum folate concentrations in unhealthy populations.

Pre-fortification							Post-fortification						
References	Blood collection period	Place	Population characteristics	n	Serum folate concentration (nmol/L)	Methods	References	Blood collection period	Place	Population characteristics	n	Serum folate concentration (nmol/L)	Methods
Other conditions							*Other conditions*						
Do Prado, D'almeida et al., 2006[17]	November 2002 to September 2003	São Paulo – SP	Children and adolescents with SLE (29 girls)	32	16.1 (± 8.0)[a]	Ionic capture (IMx System®. ABBOTT)	Chiarello, Penaforte et al., 2009[53]	2005 to 2006	Ribeirão Preto - SP	Adults with Crohn's disease	10	29.9 (± 7.9)[a]	CL (Immulite®, DPC Med Lab)
Gonçalves, D'almeida et al., 2007[19]	November 2002 to September 2003	São Paulo - SP	Girls (children and adolescents) with JIA	51	25.5 (± 10.7)[a]	Ionic capture (IMx System®. ABBOTT)	Mendes, Biselli et al., 2010[40]	February 2005 to February 2008	São José do Rio Preto - SP	Mothers of children with Down's syndrome, polymorphism DHFR ins/ins 19bp intron 1 ins/ins ins/del del/del	 27 51 27	 32.0[c] 26.9[c] 27.9[c]	CL (Immulite®, DPC Med Lab)
Vianna, Mocelin et al., 2007[52]	April 2003 to March 2005	Londrina - PR	Adults with end-stage kidney disease	93	9.3 (± 2.6)[a]	?	Minozzo, Deimling et al., 2010[42]	July 2005 to July 2006	Porto Alegre - RS	Men exposed to lead	53	14.0 (± 5.0)[a]	CL (Access Immunoassay System Beckman Instruments)
							Fialho, 2012[23]	2008	Fortaleza - CE	Children and adolescents H. pylori (+)	88	33.8 (± 8.6)[a]	CL (Immulite®, DPC Med Lab)
							Chiarani, 2012[45]	?	Porto Alegre - RS	Adults with bipolar disorder	30	24.5 (± 2.5)[a]	CL
							Santos, Scazufca et al., 2013[54]	August 2005 to April 2008	São Paulo - SP	Elderly with anemia	57	28.6 (± 13.8)[a]	CL
							De Carvalho, Muniz et al., 2013[47]	2005 to 2008	Recife - PE	Adults with NAFLD	35	34.6 (± 7.4)[a]	ECL (Elecsys®)
							Da Costa, Schtscherbyna et al., 2013[25]	2005 to 2006	Rio de Janeiro - RJ	Girls with disordered eating 11 to 14 years-old 15 to 19 years-old	 18 16	 24.9[c] 28.3[c]	RIA (Dualcount, DPC® Medlab)

HIV: human immunodeficiency virus; SLE: systemic lupus erythematosus; JIA: juvenile idiopathic arthritis; DHFR: dihydrofolate reductase; NAFLD: non-alcoholic fatty liver disease; CL: chemiluminescence; ECL: electrochemiluminescence; RIA: radioisotope assay.
[a] Serum folate concentration: mean (± SD).
[b] Serum folate concentration: geometric means (95% CI).
[c] Serum folate concentration: median.
[d] Serum folate concentration: median (P25-P75).
The missing information was represented as a question mark (?).

including the introduction of FA supplementation and the monitoring of women's health conditions, with the purpose of ensuring adequate folate blood concentrations prior to pregnancy.

One of the purposes of this review was to assess the impact of the FA fortification of wheat flour and cornmeal on serum and on red blood cell folate concentrations by comparing the pre- and post-fortification periods in Brazil. The analysis shows that most of the studies were carried out in the southeastern geographical region of the country; and there is a relative scarcity of studies covering the other regions, especially the mid-west and the northern areas; thus, the results presented herein cannot be considered to be representative of the country as a whole.

In healthy populations, an increase in serum folate concentrations was observed (57% in children and adolescents and 174% in adults). The observation that serum folate concentrations increased since fortification is a common characteristic with similar studies carried out with North American[56,57] and Chilean[58] populations. It is important to emphasize that the difference in blood folate concentrations between the pre- and post-fortification periods in Brazil may be greater than that observed in this review, since few studies that involved blood draws in the last three to four years were encountered.

Although this review presents folate concentrations (serum and red blood cell) among pregnant women and the elderly, it was not possible to make a comparison between the pre- and post-fortification values for pregnant women, due to the small number of post-fortification studies involving this cohort, but also because of the diversity of the gestational ages of pregnant women presented in these studies. It is known that there

Table 6 - Red blood cell folate concentrations in healthy pregnant women, neonates, adolescents and adults.

Pre-fortification							Post-fortification						
References	Blood collection period	Place	Population characteristics	n	Serum folate concentration (nmol/L)	Methods	References	Blood collection period	Place	Population characteristics	n	Serum folate concentration (nmol/L)	Methods
Pregnant women							*Pregnant women*						
Guerra-Shinohara, Paiva et al., 2002[11]	August to November 1999	Jundiaí - SP	Women in labor (38 to 42 weeks)	51	689 (± 311)[a]	Ionic capture (IMx System®. ABBOTT)	Guerra-Shinohara, Pereira et al., 2010[13]	February 2004 to December 2005	São Paulo - SP	Healthy pregnant women	82	1213 (917; 1396)[d]	CL (Immulite®, DPC Med Lab)
Guerra-Shinohara, Morita et al., 2004[7]	2001	Sorocaba - SP	Women in labor (38 to 42 weeks)	116	643 (591 - 701)[b]	Ionic capture (IMx System®. ABBOTT)							
Neonates							*Neonates*						
Guerra-Shinohara, Paiva et al., 2002[11]	August to November 1999	Jundiaí - SP	Blood sample from umbilical cord	48	1075 (± 400)[a]	Ionic capture (IMx System®. ABBOTT)							
Guerra-Shinohara, Morita et al., 2004[7]	2001	Sorocaba - SP	Blood sample from placental neonatal vein	116	1108 (1033 – 1188)[b]	Ionic capture (IMx System®. ABBOTT)							
Adolescents							*Adolescents*						
Do Prado, D'almeida et al., 2006[17]	November 2002 to September 2003	São Paulo – SP	Healthy children and adolescents (29 girls)	32	599 (± 246)[a]	Ionic capture (IMx System®. ABBOTT)	Almeida Dantas e De Arruda, 2010[55]	2007 to 2008	Recife - PE	Adolescents (girls)	25	1664 (± 213)[a]	ECL (Elecsys®)
Adults							*Adults*						
Barbosa, Stabler, Trentin et al., 2008[37]	2003	Sorocaba - SP	Healthy women	102	892 (807 - 987)[b]	CL (Immulite®, DPC Med Lab)	Almeida Dantas e De Arruda, 2010[55]	2007 to 2008	Recife - PE	Healthy women	335	1809 (± 364)[a]	ECL (Elecsys®)

CL: chemiluminescence; ECL: electrochemiluminescence.
[a] Red blood cell folate concentration: mean (± SD).
[b] Red blood cell folate concentration: geometric means (95% CI).
[c] Red blood cell folate concentration: median.
[d] Red blood cell folate concentration: median (P25-P75).

Table 7 - Red blood cell folate concentrations in unhealthy populations.

Pre-fortification							Post-fortification						
References	Blood collection period	Place	Population characteristics	n	Serum folate concentration (nmol/L)	Methods	References	Blood collection period	Place	Population characteristics	n	Serum folate concentration (nmol/L)	Methods
NTD and abortion							*NTD and abortion*						
Cunha, Hirata et al., 2002[48]	?	São Paulo - SP	Children with NTD, polymorphism MTHFR C677T CC CT/TT	 12 13	 760 (± 260)[a] 720 (± 180)[a]	Ionic capture (IMx System®. ABBOTT)	Guerra-Shinohara, Pereira et al., 2010[13]	February 2004 to December 2005	São Paulo - SP	Women who had spontaneous abortion	12	1145 (911; 1392)[d]	CL (Immulite®, DPC Med Lab)
Cardiometabolic alterations							*Cardiometabolic alterations*						
Uehara e Rosa, 2008[50]	2002 to 2003	Rio de Janeiro - RJ	Adults with MS: - Men - Women	 24 39	 334 (± 121)[e] 378 (± 167)[e]	RIA (Dualcount)							
Other conditions							*Other conditions*						
Do Prado, D'Almeida et al., 2006[17]	November 2002 to September 2003	São Paulo – SP	Children and adolescents with SLE (29 girls)	32	603 (± 281)[a]	Ionic capture (IMx System®. ABBOTT)							

NTD: neural tube defects; MTHFR: methylenetetrahydrofolate reductase; MS: metabolic syndrome; SLE: systemic lupus erythematosus; RIA: radioisotope assay; CL: chemiluminescence.
[a] Red blood cell folate concentration: mean (± SD).
[b] Red blood cell folate concentration: geometric means (95% CI).
[c] Red blood cell folate concentration: median.
[d] Red blood cell folate concentration: median (P25-P75).
The missing information was represented with a question mark (?).

is a reduction in blood folate from the beginning to the end of pregnancy[14,59] and, accordingly, the comparison of values among different gestational ages could result in biased data. Among the elderly, there is a lack of studies during the pre-fortification period; as only one study involving 8 individuals was found for this period, no comparison is possible.

Another point to be considered is the difference in results when different methods are used for the quantification of folate. This fact was brought to our attention in a study in which enzyme immunoassay and chemiluminescent methods were used to quantify folate concentration in pregnant women.[10] Recently, we analyzed the serum folate content in 108 samples using two methods: one microbiological method and one chemiluminescent method (Immulite® Kit, DPC Med Lab). The results showed that the two methods presented different means, with higher values of folate recorded using the microbiological method [median (25-75 percentiles): 34.7; range: 21.3-46.2 nmol/L] compared to the chemiluminescent method (median: 30.2; range: 19.3-37.6 nmol/L; Wilcoxon signed-rank test: p-value < 0.001); however, there was a significant correlation between the results of the two tests (r = 0.901; Spearman Correlation: p-value < 0.001). The different results obtained in the dosages of serum folate are the result of a lack of a specific ligand for folate or anti-folate monoclonal antibodies that could be used in the enzyme immunoassay or chemiluminescence kits.

Accordingly, if we consider the differences (14.5%) between the two methods (microbiological and chemiluminescent), this difference is much smaller than the difference found between the post- and pre-fortification periods in the groups of children and adolescents (57%) and adults (174%), leaving no doubt that there has been an increase in the concentration of serum folate since mandatory fortification.

In this review, it was not possible to evaluate the difference of red blood cell folate concentrations between the pre- and post-fortification periods, because different kits were used in the studies that evaluated similar population groups. It has already been described in the literature that different quantification methods may generate different results for red blood cell folate concentration.[60,61] It is known that TT genotype carriers of the *MTHFR* c.677C>T polymorphism present elevated red blood cell folate values compared to CC and CT genotype carriers, when folate is quantified by means of methods that use milk proteins as folate ligands (enzyme immunoassay or chemiluminescence and radio assay). However, TT genotype carriers present lower red blood cell folate values compared to other genotypes if the microbiological method is used. One possible explanation for this finding is that individuals with the TT genotype may accumulate formylated forms of folate or degradation products due to the decreased activity of the MTHFR enzyme, so that these forms may be quantified by methods that use milk proteins as ligands, rather than being quantified by the microbiological method, as they are not active forms of folate.[61]

Regarding the impact of FA fortification of flour on the rate of NTDs, several countries that have adopted the program have demonstrated a reduction in the occurrence of NTDs. In Latin America, a 33% to 59% reduction in the occurrence of NTDs has been observed.[62] Furthermore, a collaborative study conducted in Chile, Argentina and Brazil observed that the incidence of anencephaly and spina bifida per 1000 births in Brazil alone dropped from 1.12 to 0.69 and from 1.45 to 1.42, respectively.[63]

In Brazil, one study found no significant differences between the incidence of anencephaly, encephalocele and spina bifida between the two periods;[64] another study found a significant reduction (39%) in the incidence of spina bifida.[65] Recently a transversal study has shown that the incidences of anencephaly and spina bifida were reduced by 22% and 48%, respectively, with no reduction in the incidence of encephalocele in municipals of the state of São Paulo following mandatory fortification. In total, the incidence of NTDs has dropped 35%, from 0.57 to 0.37 cases per 1000 live births.[66] Besides these studies, a systematic review in nine countries (Brazil, Chile, Argentina, Canada, the USA, Costa Rica, Iran, Jordan and South Africa) observed that the FA fortification of foods has had a considerable impact, with reductions in the incidence of NTDs varying between 15.5% and 58.0%.[67]

Another way of evaluating the impact of fortification is by means of dietary folate intake, such that a significant decline in the rate of inadequate folate intake has been observed in the countries that have adopted mandatory FA fortification.[68-70] In Brazil, transversal studies have shown inadequate folate intake among pregnant women,[71-73] teenagers[74] and adults[75] in the pre-fortification period. However, in the post-fortification period, no inadequate folate intake has been observed among pre-school children.[76] An inadequate intake of FA was observed in 15.2% adolescents in the town of Indaiatuba (state of São Paulo).[21]

Finally, one factor that must be taken into consideration when evaluating the FA fortification of flour is the level of compliance with legislation by flour mills. ANVISA RDC Resolution no. 344 mandates the addition of 150 µg of FA to every 100 g of wheat flour and cornmeal; however, a maximum limit for the quantity of FA has not been established. Non-compliant FA concentrations regarding RDC no. 344 have been observed in cornmeal (from 96 to 558 µg per 100 g) and in wheat flour (73 to 233 µg per 100 g).[77] Since both lack and excess of folate can be harmful, these data emphasize the need for constant monitoring of the FA content in flour products by health authorities, especially as several studies have observed supraphysiological concentrations of this vitamin (serum folate > 45 nmol/L) among several populations. In conclusion, the studies show an increase in the serum concentrations of folate and a reduction in the incidence of NTDs in Brazil. However, national wide-range evaluations are necessary, in order to be able to monitor blood concentrations in the Brazilian population and the FA content of fortified foods.

Conflicts of interest

The authors declare no conflicts of interest.

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