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ORIGINAL ARTICLE

An overview of folate status in a population-based study from São Paulo, Brazil and the potential impact of 10 years of national folic acid fortification policy

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BACKGROUND/OBJECTIVES: Food fortification is an important strategy in public health policy for controlling micronutrient malnutrition and a major contributing factor in the eradication of micronutrients' deficiencies. Approximately 50 countries worldwide have adopted food fortification with folic acid (FA). FA fortification of wheat and maize flours has been mandatory in Brazil since 2004. To assess the effect of 10 years of FA food fortification policy on folate status of residents of São Palo, Brazil using a population-based survey.
SUBJECTS/METHODS: Data were from 750 individuals aged ⩾12 years who participated in a cross-sectional population-based survey in São Paulo city, Brazil. Fasting blood samples were collected, and folate was assayed by affinity-high performance liquid chromatografy method with electrochemical detection. The participants provided information about food intake based on two 24 h dietary recall.
RESULTS: Only 1.76% of population had folate deficiency (< 6.8 nmol/l). The mean folate concentration was 29.5 (95% confidence interval: 27.3–31.7) nmol/l for all sex–age groups. The mean folate intake for the population was 375.8 (s.e.m. = 6.4) μg/day of dietary folate equivalents (DFEs). When comparing folate intake in DFE from food folate and FA from fortified foods, FA contributed 50% or more of the DFE in almost all sex–age groups. The major contributors of folate intake are processed foods made from wheat flour fortified with FA, especially among subjects younger than 20 years old.
CONCLUSIONS: The deficiency of folate is very low, and food fortification contributed to folate intake and had a notable influence on rankings of food contributors of folate.

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INTRODUCTION

The importance of folate in maintaining healthy reproduction has been known for decades.[1] The original rationale for this knowledge was the observation that low maternal intake of folate is also associated with a higher risk of giving birth to newborns with serious congenital abnormalities of the central nervous system, that is, neural tube defects (NTDs). The hypothesis of relationship between folate deficiency and occurrence of NTDs was mentioned in 1965.[2] Since then, many studies have documented the protective effect of folic acid (FA) supplementation on the occurrence of NTDs.[3–5]

In addition, food fortification has a long history of use as a strategic program in public health policy for controlling micronutrient malnutrition in several countries, and fortification is a major contributing factor in the eradication of diseases associated with micronutrients' deficiencies, as in the case of folate deficiency.[6] In a further effort to increase folate intake, improve folate status and reduce the occurrence of folate-preventable NTDs, mandatory FA food fortification has been adopted in almost 50 countries worldwide.[7] The United States and Canada were the first countries to add FA to enriched grain products in 1998.[8,9] In South America, mandatory FA fortification programs started in Chile in 2000, followed by those in Brazil in 2002 and in Argentina in 2003.[10–12] The Brazilian Ministry of Health has required FA fortification of wheat and maize flours (150 μg of FA per 100 g of flour) since December 2002 with mandatory compliance by June 2004.[11]

The effectiveness of these FA fortification programs in increasing folate status and the consequent prevention of NTDs has already been reported by several studies.[13,14] More recently, studies have described potential effects associated with consumption of FA such as cancer and unmetabolized FA in blood masking vitamin B12 deficiency.[15,16] Thus, recommendations for monitoring and evaluating FA intake and folate status are necessary at the population level to ensure the safety of FA fortification, particularly in countries that mandate FA fortification of food products.[7]

The aim of this study was to assess folate status in the general population after 10 years of mandatory FA fortification of wheat and maize flours, and describe dietary intake and major contributors of folate. Currently, this is the only representative data on folate concentration available from Brazilian population.

MATERIALS AND METHODS

Study population

The study population was selected from the 'Health Survey—São Paulo', a cross-sectional study of health and living conditions among a representative sample of individuals living in São Paulo, Brazil, in 2008.

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The study had complex design. It defined eight age domains as follows: < 1 year old, 1–11 years old, and three more age groups for each gender —12–19 years (adolescents), 20–59 years (adults) and 60 years or over (elderly). Two-stage cluster sampling of census tracts and households was performed based on PNAD 2005 (National Household Sample Survey 2005) and included only urban areas. A total of 3271 individuals (197 < 1 year, 383 1–11 years, 605 12–19 years, and 2086 20 years and older) participated in the Health Survey—São Paulo. Of these, 750 individuals, 12 years and older, donated a blood sample and completed the dietary measurement. The study population included all participants with complete dietary intake data and sufficient blood sample for analysis of biochemical parameters.

The sampling weights were constructed for each participant considering the complex sample design, the adjustment for non-response, and adjustment for female and male, different ages, to provide the equilibrium between the demographic characteristics of the sample to assure that the sample represented the population. More details about the plans and operations of this survey may be found elsewhere.[17]

The study project was approved by the Ethics Committee at the School of Public Health, University of São Paulo (approval number: #2001). All participants were registered in the study after signing the consent forms at the beginning of the first visit. The guardians were responsible for signing the consent forms if participants were younger than 18 years.

Dietary assessment

Two multiple pass 24 h dietary recalls were performed to measure the dietary intake. The first 24 h was collected during in-home interview by trained interviewers, and the second 24 h was collected by multiple pass by phone. The multiple pass method considers five steps to collect the individual's dietary intake.[18] The 24 h covered all the days of the week and seasons. Foods reported in both 24 h were identified, quantified and converted into energy and nutrient using Nutrition Data System for Research software version 2007 (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, USA), which is based on the data from food composition table published by the US Department for Agriculture. We emphasize that Brazil does not have any database on food composition that analyzed food folate and its different forms—that is, synthetic and food folate. Therefore, the researchers selected the food composition table from the US Department for Agriculture because this table presents the folate levels as dietary folate equivalents (DFEs), differentiating between the bioavailability of folate that is contained naturally in foods (food folate) and the synthetic form (folic acid) that is added to fortified products, and the researchers made all corrections needed to get the correct information about total folate (μg of DFE), FA and food folate considering the Brazilian fortification. Thus, the amount of FA and DFE levels were corrected to account for the level of fortification of wheat and maize flour that has been mandatory in Brazil since 2004. There is a difference between the amounts of FA added to fortified foods in Brazil (150 μg/100 g of flour) and in the United States (140 μg/100 g of flour).

The prevalence of inadequate folate intake was calculated as the proportion of healthy individuals with usual intake below the estimated average requirement according to the age and sex groups. Confidence intervals for the prevalence of inadequate intakes were derived from s.e. based on a jackknife replication technique considering the complex sample design using the PC-SIDE software (version 1.0, Ames, IA, USA) for intake distribution public health nutrition estimation (PC-SIDE). The contribution of each food group to folate intake was obtained considering the total amount of the component provided by the food group for all individuals, total folate intake from all food groups for individuals and the sample weight for that individual.[19]

Blood analyses

The blood samples were collected through venipuncture in tubes containing EDTA (ethylenediaminetetraacetic acid) after 12 h of fasting. The tubes were immediately centrifuged followed by processing into aliquots of plasma and storage at − 80 °C. Plasma folate concentrations were determined with the use of the affinity-high performance liquid chromatografy (HPLC) method with electrochemical detection.[20]

Statistical analysis

All analyses were carried out using STATA (release 13.0, StataCorp LP, College Station, TX, USA). Sampling weights were used to generate proportions and means that are representative of the population from São Paulo city, Brazil.

Mean values, s.e. and 95% confidence intervals (CIs) were calculated considering the predicted usual intake distribution by multiple source method, which is a useful and applicable statistical technique to estimate usual nutrient intake distributions attenuating the effects of within-person variation, if at least two repeated dietary measurements per participant are available.[21] Differences between means of folate concentration were analyzed using the Wald test, which accounts for the weights from complex samples; and differences in the proportions of folate deficiency by sex and age groups were evaluated with Pearson's χ^2-test. All tests were two-tailed, $\alpha = 0.05$.

RESULTS

Women accounted for 53.1% of the population. Average age was 40.6 (95% CI: 38.8–42.5) years, and 20.3% were current smokers. Nutritional status based on body mass index classification indicated that 47.9% had excess weight (body mass index $\geqslant 25\ kg/m^2$), and 2.7% of the population used vitamin B supplements, which included folate.

The mean plasma concentration of total folate was 29.5 nmol/l for all age and sex groups. The fifth and 95th percentiles for plasma folate in the total population were 9.6 and 64.5 nmol/l, respectively (Table 1). We observed differences in mean folate concentrations between the sex and age groups. Sex differences in folate status were observed in adults (20–59 years), such that women had significantly higher mean plasma folate than males had ($P = 0.038$). Also, elderly women aged 60+ years had higher

Table 1. Means, s.e.m., 95% CIs and distribution percentiles for plasma folate concentration (nmol/l) by sex and age groups

	Folate concentration (nmol/l)														
	Reference group	*n*	*Mean*	*s.e.m.*	*95% CIs*		*Fifth*	*10th*	*25th*	*50th*	*75th*	*90th*	*95th*	*P-value (group)*[a]	*P-value (group)*[b]
Total		710	29.5	1.1	27.3	31.7	9.6	12.7	18.7	27.4	39.0	53.1	64.5		
Males		299	27.4	1.6	24.3	30.5	9.0	11.7	17.9	25.5	37.9	50.5	58.7	0.035*	
12–19 years	1	85	27.9	1.7	24.5	31.3	8.3	9.8	16.7	24.6	33.3	48.6	58.3	0.63 (1–1)	0.47 (1–2)
20–59 years	2	107	26.0	1.9	22.0	29.9	11.6	13.9	17.4	24.0	35.2	45.5	48.6	0.038 (2–2)*	0.004 (2–3)*
60+ years	3	107	34.6	2.6	29.5	39.8	10.0	12.3	20.2	31.0	44.7	58.7	67.2	0.68 (3–3)	0.033 (1–3)*
Females		411	31.4	1.3	28.9	33.9	9.7	14.5	19.2	28.0	39.7	54.6	67.6		
12–19 years	1	69	26.7	2.1	22.5	30.9	7.4	9.6	17.7	24.1	33.0	45.7	55.7		0.09 (1–2)
20–59 years	2	179	30.9	1.5	27.9	34.0	9.7	14.3	18.8	27.9	39.4	49.6	62.8		0.046 (2–3)*
60+ years	3	163	36.1	2.4	31.3	40.8	11.9	16.0	22.4	29.6	44.0	64.5	81.7		0.004 (1–3)*

Abbreviation: 95% CIs, 95% confidence intervals. [a]Wald test (difference between plasma folate by sex, *considering P < 0.05 significant). [b]Wald test (difference between plasma folate by age group, *considering P < 0.05 significant).

plasma folate than adolescent women aged 12–19 years ($P = 0.004$) and adult women aged 20–59 years ($P = 0.046$). In males, age differences were noted in the same age groups; elderly had higher mean folate than adolescents ($P = 0.033$) and adults ($P = 0.004$) had. Figure 1 shows the proportions of the population by sex and age groups with clinical folate deficiency (< 6.8 nmol/l). Of the total population, 1.76% (95% CI: 0.93–3.30) present serum folate deficiency, and 2.85% (95% CI: 0.95–8.23), 1.50% (95% CI: 0.60–3.69) and 2.09% (95% CI: 0.81–5.32) have deficiency in adolescents, adults and the elderly, respectively. No differences were observed in the proportions of folate deficiency between males and females, nor among adolescents, adults and the elderly.

As shown in Table 2, overall mean folate intake was 375.8 (s.e.m. = 6.4) μg/day of DFE. The highest mean was observed in adolescent males, which was 466.2 (s.e.m. = 14.6) μg/day. The lowest mean folate intake was observed in women over 20 years old, ranging from 314.5 (s.e.m. = 8.1) in women between 20 and 59 years to 337.3 (s.e.m. = 7.6) μg/day for women over 60 years old. When comparing various sources of folate intake, that is, food folate and FA from fortified foods, the percentage of vitamin intake from FA contributed 50% or more of the daily DFE in almost all sex–age groups. Only the elderly consumed more DFE from food folate than FA. The prevalence of inadequate folate intake was higher in women than in men.

Table 3 displays the rank of major contributors of folate intake, considering the percentage of contribution by 10 food groups for the age group. Bread, rolls and beans accounted almost 50% of folate intake in all age groups. The major contributors of folate intake are food items made from enriched wheat flour (bread and rolls, pasta, crackers, cookies with filling, pizza, sandwiches, cakes and baked pastries, and salty snacks). In adolescents, only 2 of 10 food groups are comprised of foods that contain the naturally occurring form of the vitamin (beans and meat), while elderly has six food groups (beans, vegetables, fruits and fresh juice, soups, milk and liver).

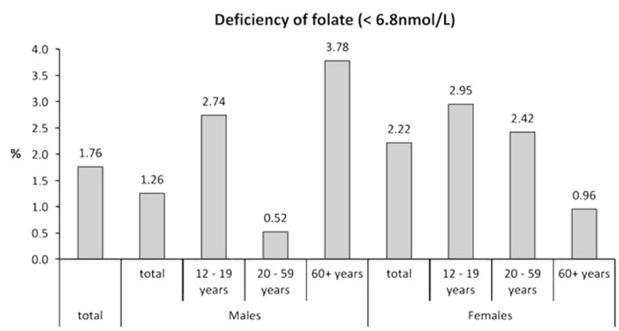

Figure 1. Proportion of deficiency of folate in plasma (< 6.8 nmol/l) by sex and age groups.

DISCUSSION

The goal of this study was to describe the folate status of residents of São Palo, Brazil and to assess the proportion of population who had folate deficiency after fortification. Only 1.76% of population residents in São Paulo had plasma folate concentration < 6.8 nmol/l (< 3 ng/ml), cutoff for deficiency, according to our results, and prevalence of inadequate folate intake was higher in women than in men after 10 years of FA mandatory fortification of flours in Brazil. The major contributors of folate intake were processed foods made from wheat flour fortified with FA, particularly in the young population.

Our results showed that plasma folate deficiency is very low in this population, 1.76% of all population had folate deficiency and the mean plasma concentration of total folate was 29.5 nmol/l after mandatory fortification. Regarding the comparison of folate levels before and after fortification, Brazil lacks biochemical data assessing the folate status before or after fortification in the same sample, in national or even regional populations. Only one study presented this outcome. The study was conducted in Brazilian women ($n = 99$), patients of the Nutritional Ambulatory Care of the Federal University of Rio de Janeiro, and showed no difference in mean of plasma folate between the two periods, 15.7 and 12.1 nmol/l, before and after fortification, respectively. They found that plasma homocysteine level was reduced after the fortification.[22] Another relevant study was a review, published in 2014, which investigated the impact of the FA fortification of flours on serum and red blood cell folate concentrations. It utilized data from almost 50 Brazilian studies in different populations from the pre- and post-fortification period. After analyzing the studies, the researchers concluded that serum folate concentrations increased by 57% in children and in adolescents, and almost doubled in adults after the implementation of flour fortification with FA in Brazil. The range of mean serum folate in healthy adults was from 8.8 to 19.2 nmol/l (before fortification) and from 14.4 to 35.3 nmol/l (after fortification) among the study populations selected.[23] The researchers conducted the analysis comparing the outcomes of pre- and post-fortification period from samples of

Table 2. Means, s.e.m., 95% CIs and prevalence of inadequacy for dietary folate intake by sex and age groups

	n	*DFE (μg/day)*			*Food folate (μg/day)*				*Folic acid (μg/day)*				*EAR (μg/day)*	*Prevalence of inadequacy*		
		Mean	*95% CIs*		*Mean*	*95% CIs*		*% DFE[a]*	*Mean*	*95% CIs*		*% DFE[a]*		*%*	*95% CIs*	
Total	750	375.8	363.0	388.6	174.5	167.2	181.8	47.7	117.9	112.6	123.2	52.9				
Males	314	418.3	397.5	439.2	194.7	181.4	208.1	47.8	130.1	122.4	137.8	52.6				
12–19 y	88	466.2	436.8	495.6	193.6	182.1	205.0	42.8	159.9	146.8	173.1	57.6	330	4.0	0.0	12.3
20–59 y	113	417.7	387.4	447.9	195.7	176.1	215.3	48.0	129.0	119.1	138.9	52.5	320	17.4	0.0	36.4
60+ y	113	376.6	350.2	403.0	190.7	178.3	203.1	51.5	107.6	97.4	117.7	48.4	320	9.5	0.0	28.7
Females	436	338.3	325.6	351.0	156.7	151.8	161.6	47.6	107.2	101.5	112.9	53.1				
12–19 y	70	384.9	356.1	413.7	159.2	146.5	171.8	42.2	130.6	119.8	141.5	57.4	330	25.0	10.7	39.2
20–59 y	190	337.3	322.0	352.6	155.2	148.9	161.4	47.2	108.0	100.6	115.4	53.7	320	34.7	12.7	56.7
60+ y	176	314.5	298.3	330.7	160.9	152.2	169.5	52.2	90.5	83.5	97.5	48.3	320	43.9	27.2	60.5

Abbreviations: EAR, estimated average requirement; DFE, dietary folate equivalent; 95% CIs, 95% confidence intervals; y = years. [a]% of food folate in DFE or folic acid in DFE considering the quantity of DFE occurring naturally in food equals the μg of folate as reported, and the dietary folate equivalents provided by fortified foods equal the μg of food folate plus 1.7 times the μg of added folic acid.

Table 3. Major contributors of dietary folate intake by age group

Adolescents				*Adults*				*Elderly*			
		Percentage (%)				*Percentage (%)*				*Percentage (%)*	
Rank	*Food*	*Relative*	*Cumulative*	*Rank*	*Food*	*Relative*	*Cumulative*	*Rank*	*Food*	*Relative*	*Cumulative*
1	Bread and rolls	35.2	35.2	1	Bread and rolls	32.5	32.5	1	Bread and rolls	33.9	33.9
2	Beans	10.9	46.2	2	Beans	10.6	43.2	2	Beans	9.4	43.3
3	Pizza	8.0	54.2	3	Pasta	8.9	52.1	3	Vegetables	8.8	52.1
4	Pasta	7.2	61.4	4	Pizza	7.5	59.6	4	Fruits and fresh juice	7.6	59.7
5	Cookies with filling	5.1	66.5	5	Mixed dishes	4.6	64.3	5	Pasta	6.5	66.2
6	Crackers	3.9	70.3	6	Meat	3.6	67.9	6	Cakes and baked pastries	3.4	69.6
7	Salty snacks	3.6	74.0	7	Vegetables	3.2	71.1	7	Crackers	3.4	73.0
8	Meat	3.5	77.5	8	Fruits and fresh juice	3.1	74.2	8	Soups	3.0	76.0
9	Sandwiches	3.3	80.8	9	Crackers	2.6	76.9	9	Milk	2.7	78.7
10	Cakes and baked pastries	2.4	83.2	10	Cakes and baked pastries	2.2	79.0	10	Liver	2.6	81.3

different studies. Importantly, the review considered studies with different methods for the quantification of folate concentration.

Other countries that have implemented mandatory FA fortification have reported comparisons of pre- and post-fortification folate levels in the population. One study analyzed national data from the US population in pre- and post-fortification periods. Although they used higher cutoffs (< 10 nmol/l) than the values of 6.8 nmol/l, the prevalence of low serum folate concentrations during post-fortification was very low ($\leqslant$1%) versus 24% during pre-fortification. The serum folate levels were of 16.7 nmol/l (before fortification) and 41 nmol/l (after fortification), regardless of demographic subgroup.[24] In Canada, the biochemical evidence showed that no women of childbearing age were folatedeficient in the post-folic acid fortification era.[25] The results obtained from another study in Canada reported that the mean serum folate concentration increased after fortification, from 14.8 to 24.2 nmol/l, among $> 15\,000$ women aged 65 years.[26] Similar findings are found in Chile in South America, where folate deficiency (serum folate < 3.2 nmol/l) was not observed in women of childbearing age in the post-fortification era. Prior to fortification, the mean serum concentrations were 9.7 nmol/l compared to 37.2 nmol/l, after fortification in Chile.[27]

The FA fortification program contributed to increased nutrient intakes. Overall, findings from National Health and Nutrition Examination Survey 2003–2006 in the United States showed that the food fortification was a key contributor to folate sufficiency, and, along with nutrient supplements, it helped to reduce the percentage of the population who consumed less than the estimated average requirement. Only 10.7% of population who consume fortified foods, and 7.6% of population who consume both fortified foods and dietary supplements failed to meet the estimated average requirement for folate intake in population aged $\geqslant$2 years, compared to 88% of the population who consume only naturally occurring food contributors of folate.[28] In Brazil, a previous study assessed the prevalence of inadequate folate intake comparing pre- and post-fortification of flours with FA, and it reported an important reduction in the prevalence of inadequacy in all groups, especially adolescent and adult males, 72–$<$1% and 76–6%, respectively, but the prevalence of inadequacy remained high in adult women (38%).[29] Likewise, results from our study reported similar results with high prevalence of inadequate intake among adult and elderly women. It is important to emphasize that measuring the folate quantity from foods is difficult, and assessing the prevalence of folate deficiency in a population comparing usual dietary intakes with estimated requirements needs to be carefully discussed.[6] In addition, our dietary assessments in this study were limited, as under-reports were not calculated, and the results can be underestimated or overestimated.

It is clear that the addition of FA to flours increased folate intake. As noted earlier, without added nutrients, a high percentage of the entire population had inadequate intakes of numerous micronutrients.[28,30] In the United States, different sources provide FA such as dietary supplements, enriched cereal grain products, ready-to-eat breakfast cereals and other types of fortified food.[30,31] Foods such as lentils, dried beans, peas, and dark green vegetables such as broccoli, spinach, collard greens or turnip greens are rich in naturally occurring folate.[31] At least 50% of the US population aged $\geqslant$2 years,[26] and $<$50% of children and adolescents between 2 and 18 years of age[30] consume food folate from this group. In American diet, the main foods that contribute to folate intake after fortification are ready-to-eat cereal in children and in adolescents[30] and bread, rolls and crackers in adults.[32] In Brazil, we observed that total folate intake was obtained from two different sources—food folate and fortified foods. FA from fortified foods contribute 50% or more of the DFE in almost all sex–age groups (mean of 52.9% in all population), except among the elderly. The largest contributor to folate intake was bread, but we also noted that beans, which contain the natural form of the vitamin, remained an important source of folate, even after the mandatory fortification of flours.

Interestingly, we observed higher folate levels in the elderly when compared to adolescents and adults, and 6 of 10 food groups had relevant contributions to the naturally occurring form of the vitamin. FA has better bioavailability than food folate[33] and could increase folate status; folate intake is calculated considering the FA recommended by Brazilian legislation (150 μg of FA per 100 g of flour).[11] However, the Brazilian authorities have not routinely supervised the amount of FA added in flours to certify whether the correct amount of the vitamin has been added. Two studies analyzed samples of corn and maize flours and found inadequate FA in the samples. FA content ranged from 73 to 558 μg of FA per 100 g of flour.[34,35] Therefore, to consider only dietary intake can lead to erroneous conclusions about folate status. Nevertheless, continued monitoring of top food contributors is important to determine dietary sources of the nutrient and can help provide dietary advice considering regional differences of the population.

Another relevant issue in folate intake is dietary supplement use. The monitoring of folate deficiency without inclusion of this important contributor to folate status is inadequate. In contrast with other countries where dietary supplement is often used,[36,37] Brazil does not have national data regarding dietary supplements, and a previous Health Survey in São Paulo reported very low prevalence of supplement use (6.35%).[38] We found that 2.67% of the population used vitamin B supplements, which include folate. We did our analysis including and excluding the supplements users, and no difference was found in the results. Therefore, we decided to include all individuals in the analyses.

Although the present study did not evaluate the occurrence of NTDs, a literature review has indicated that historically, FA fortification has been shown to be effective in decreasing the incidence of NTDs. In the United States, the prevalence of NTDs reported on birth certificates decreased from 37.8 per 100 000 live births before fortification to 30.5 per 100 000 live births conceived after mandatory FA fortification, representing a 19% decline.[13] In South America, some researchers estimate that a reduction in 52 types of congenital anomalies is associated with FA fortification programs. The study analyzed data from ECLAMC (Spanish acronym for Latin American collaborative study of congenital malformations), a study across three Latin American countries (Argentina, Chile and Brazil) where FA fortification has been implemented. They detected a 30–50% decrease in the rates of the selected anomalies.[39] Nevertheless, some researchers discuss that the magnitude of the decrease in NTD prevalence reported in each country has been dependent on a number of factors since the initiation of food fortification with FA. Thus, they mentioned that it is necessary to consider factors such as folate status of the population before fortification, the number of individuals who consume fortified foods, background prevalence of NTD and the capacity of birth defects surveillance systems to determine the decline in prevalence of NTDs resulting of FA fortification.[14,40]

In summary, many countries that have food FA fortification have successfully improved folate status in their populations. The studies conducted especially in the United States and Canada have showed that the fortification effectively increased FA intake[41] and folate status,[42] and the incidence of NTDs decreased substantially after fortification.[13,43] But this success has been a result of a higher intake of FA and some researchers are concerned that this increased FA intake is more than expected.[44,45] Although no evidence exists to suggest that this higher intake of FA may injure the population, concerns have been raised regarding the safety of high exposure to FA through the combination of fortified foods and supplements.[40,44] It is emphasized that the use of supplements is low in Brazil.[38] However, many articles have been written describing some adverse effects associated with high folate intake. Some studies suggest that high oral doses of FA bypass the normal folate metabolism, resulting in presence of unmetabolized FA in the serum. There are no known biological effects of unmetabolized FA. However, unmetabolized FA may be a contributing factor in safety concerns associated with high oral doses of FA.[46,47] In addition, increased FA intake and the consequent increase in serum folate could mask vitamin B12 deficiency, delaying the diagnosis of deficiency or even exacerbating its neurologic manifestations.[48] Moreover, concerns about risk of certain types of cancer have been raised, and the evidence is most persuasive for colorectal cancers.[49] This issue is particularly controversial because there is little observational data and results are inconclusive; other experimental and long-term follow-up studies are needed.

In conclusion, the deficiency of folate was very low, and FA fortification of flours contributed to increased folate intake and had a notable influence on rankings of food contributors of folate in this Brazilian population. Importantly, this shows that strategy of food fortification with FA can lead to improvements in the folate status of a population. As the benefits are potentially large, food fortification can be a very cost-effective public health intervention. Importantly, further research is necessary to clarify the long-term effects of higher intake of FA and to elucidate these complex relationships with potential adverse effects. It is imperative to continuously monitor the folate status and intake in the population to guarantee the safety of exposure to FA, especially in countries with mandatory FA fortification.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

JS, RMF and DMLM designed the research and interpreted the data; JS, DMLM, JSe, LP and CR conducted the research; JS and DMLM analyzed and wrote the paper, and had primary responsibility for the final content. All authors read and approved the final manuscript.

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