Recommendations for folate intake in women: implications for public health strategies

Recomendações para ingestão de folato por mulheres: implicações para as estratégias de saúde pública

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Abstract

Folate deficiency has been associated with anemia and other adverse outcomes in pregnancy such as neural tube defects. The current recommendations for prevention of such outcomes are difficult to achieve through diet only, and folic acid supplementation and food fortification are feasible public health strategies. However, it is necessary to determine the usual diet and supplement use among women of reproductive age, including an accurate assessment of other dietary micronutrients. In addition to the beneficial effects observed in randomized clinical trials, health risks to the population have also been widely evaluated and discussed in the scientific community: for a minority to benefit from fortification programs, many are exposed to high folic acid intake levels.

Folic Acids; Pteroylpolyglutamic Acids; Nutritional Epidemiology

Introduction

Data released by the World Health Organization (WHO) show that women of reproductive age, along with infants younger than 2 years of age, are at their nutritionally most vulnerable stage in life [1]. In contrast to trends in developed countries over the past 70 years, high rates of maternal morbidity and mortality persist in developing regions, particularly among low-income women. Maternal mortality figures differ greatly between wealthy and poor nations, with the later accounting for 90% of such deaths [2]. Around 160 million women become pregnant ever year, approximately 15% of whom develop serious preventable complications, and millions of newborns do not survive the first week of life due to the absence or lack of proper prenatal healthcare [3].

One of the indirect complications contributing to maternal health (and consequently to infant health) is maternal anemia. Anemia is especially common among women of reproductive age secondary to menstrual bleeding and to physiological changes during pregnancy [4], yet remains prevalent owing to limited availability of diagnostic and treatment services.

From a nutritional standpoint, deficiencies in iron, vitamins B12, A and folate cause hematologic changes which if untreated can evolve to an anemic state [5]. Folic acid has been a focus of research for the past 30 years given its role in the

prevention of another condition: neural tube closure defects.

The role of folic acid in biochemical reactions, such as those involved in metabolism of aminoacids and in the synthesis of DNA, renders it a critical nutrient in embryogenesis. During embryogenesis closure of the neural tube, the structure from which the brain and spinal column are derived, takes place [6]. Neural tube closure defects can lead to death of the infant or to serious life-long complications. There is evidence for a protective effect against these defects through pre and periconceptional folate supplementation [7].

Folic acid supplementation in women was therefore introduced to address two principal problems: maternal anemia and neural tube closure defects. In view of limited adherence to supplementation however, food fortification was subsequently proposed for prevention of neural tube closure defects. The aim of the present study was to consider both these outcomes by analyzing and summarizing the recommendations and conducts pertaining to consumption of this vitamin in light of the latest scientific evidence.

Definitions and background

Although the umbrella term "folic acid" has been adopted in this article to denote all forms of the vitamin, including those found in plasma and erythrocytes, there are key differences between folate (occurring naturally in foods) and oxidized folic acid (synthetic form used in supplements and fortified foods). Folate is in the form of polyglutamates (pteroylpolyglutamate) whereas folic acid is in monoglutamate (pteroylmonoglutamate) form. Polyglutamates have higher metabolic activity and are better retained by cells, while monoglutamates pass through cell walls more rapidly. In humans, the metabolism of polyglutamates requires their deconjugation to monoglutamates within enterocytes, explaining their low bioavailability (~50%) compared to monoglutamates (~85%) [8]. Moreover, anti-folate components present in vegetables as well as exposure to heat and light during cooking and storage, all contribute to lowering folate availability [9].

Differences in bioavailability hamper the establishing of recommendations. To adjust for the bioavailability differences between folate and folic acid, Dietary Folate Equivalents (DFE) are used. The DFE assumes that the bioavailability of folic acid added to foods is 1.7-fold greater than that of natural folate (mathematically, DFE = folate + 1.7 x folic acid) [10]. An individual's folate acid status therefore depends on the source of the vitamin in the diet as well as the absolute amount consumed.

Folic acid supplementation was primarily indicated for the prevention and treatment of megaloblastic and maternal anemia [11]. Supplementation is also indicated in individuals with a genetic defect requiring greater amounts of folate than can be derived from the diet [12]. Based on evidence that folic acid also prevents neural tube closure defects [13], supplementation in public health programs was recommended, particularly since, unlike megaloblastic anemia, sera concentrations of folic acid in mothers of children with neural tube defects were normal [14]. Against this background, the present article discusses the importance of recommendations of folic acid in public health programmes for the prevention and control of two different outcomes, namely: anemia during pregnancy and neural tube closure defects.

Anemia during pregnancy

Around one million maternal and infant lives are cut short every year due to anemia, a condition associated to low birth weight, prematurity and neonatal mortality, and a cause of disablement, mental retardation and compromised work performance [15].

Anemia has multiple causes including blood loss, parasitic infections, cancer, hemoglobinopathies, malaria and deficiencies in nutrients such as iron, vitamins B12, A, riboflavin and folic acid [15]. The contributions of nutritional factors to the etiology of anemia can vary according to age, physiological state and socio-economic conditions, such that interpretation of a specific marker in isolation is not valid in scenarios where the etiology of anemia may be more complex.

The WHO considers anemia a global public-health problem: a significant proportion of women of reproductive age (29.6%) live in countries where anemia is a serious health problem (anemia prevalence ≥ 40%), and over half the global population of pre-school children (56.3%) and pregnant women (57.5%) reside in such countries, the majority of which are located in Africa, Asia and Latin America [16]. Studies on the prevalence of folic acid deficiency and also on the hematological response to folic acid in Brazil are scarce [17]. According to Metz [18], anemia due to folic acid deficiency in developed countries is rare, but still occurs in developing countries, especially in areas endemic for malaria and with a high incidence of iron deficiency anemia, hemoglobinopathies and HIV.

Population-based data for Brazil reveals that the occurrence of anemia among women of re-

productive age (10-49 years) ranges from 15.2% (rural zone of Pernambuco) to 26.2% (Piaui), and from 14.7% (Rio de Janeiro) to 40.4% (Recife, Pernambuco) among pregnant women [19]. In São Leopoldo, Rio Grande do Sul, a transversal study on a representative sample of 312 women (20-60 years) found an overall prevalence of 19.2%, and double this rate among negro women (54%) [20]. There is a lack of representative data for other regions in Brazil for the past ten years estimating the magnitude of the problem in women of reproductive age.

The Department of Nutrition for Health and Development of the WHO summarized the current recommendations for creating and implementing public health intervention programmes [21]. Weekly iron-folic acid supplementation, in synchrony with enterocyte turnover, was proposed as an equally effective preventive measure as daily supplementation in public-health programmes, and a more optimal approach given its lower side-effects and ease of administration in community settings [22]. It is important that an assessment is carried our prior to commencing interventions and once running, programmes should be monitored on a monthly basis to check processes and outcomes throughout the first year, and then verified every five years thereafter.

In Brazil, the goal of the National Programme of Supplementation with Iron, proposed by the General Coordination of Food and Nutrition Policy (CGPAN), is to reduce the prevalence of anemia in infants aged 6-18 months, in pregnant women and women after child birth or miscarriage [23]. This is the first Brazilian programme of its kind to consider both supplementation with iron and provision of folic acid to target groups: 5mg/day of the vitamin administered from the 20th week of pregnancy up until childbirth. According to the guidelines of the Institute of Medicine, the recommended dose to prevent anemia in pregnant women is 0.4mg/day of folic acid (diet + supplement) [24], with a maximum safe dose of 1mg/day [10]. Given the known and unknown adverse effects of over-supplementation with this vitamin, further studies reassessing this programme are needed.

Table 1 contains the recommendations for iron and folic acid supplementation in women for anemia prevention.

Neural tube closure defects

The neural tube normally closes within four weeks of conception or two weeks after interruption of menstruation. Cases of neural tube closure defect can lead to the pregnancies ending in miscarriage, stillbirth or in a child who may experience life-long medical complications [25]. Genetic, nutritional and environmental factors, or a combination of these, play a definitive role in the development of these conditions.

• Genetics

Blom et al. [26] proposed the theory of methylation, according to which problems in the methylation of lipids, DNA and protein during embryogenesis translate to a higher risk for neural tube defects in individuals with specific mutations. The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) catalyzes the irreversible reduction of 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, a methyl donor for biosynthesis of methionine and nucleotides. Mutations in MTHFR have been associated to greater risk of neural tube defects in some populations [27] yet not in others [28]. Other genes may influence the methylation cycle either through remethylation (MTR, MTRR, BHMT and BHMT2) or by transsulfuration (CBS) of homocysteine, compromising methylation and increasing homocysteine concentrations which are in turn associated to greater risk of neural tube defects [29]. A second hypothesis centers on the genes codifying to enzymes needed for nucleotide biosynthesis: there is evidence that a polymorphism in methylenetetrahydrofolate dehydrogenase (MTHFD1) is associated with a higher risk of neural tube defects in Italian [30], but not English [31], populations. The third theory concerns the genes codifying proteins involved in the transport, capture and cell retention of folate, such as the reduced folate carrier (RFC), folyl-gamma-glutamate carboxypeptidase (GCP2) and folylpolyglutamate synthase (FPGS), although not all studies have confirmed neural tube defect risk [32].

• Nutrition

After the identification of folic acid as an important vitamin in the occurrence/reoccurrence of neural tube defects [33], numerous studies ensued assessing associations between diet and neural tube defects. Folic acid acts as a donor of methyl groups in the synthesis of DNA, and the relationship between folic acid deficiency and neural tube defects may be linked to lower gene expression, given that methylation of DNA influences transcription and genomic stability [34]. Gene-nutrient interaction has been observed in recent studies: the effect of the mutant homozygosis of the MTHFR C677T polymorphism on homocysteine concentrations, a risk factor for neural tube defect, was reduced by moderate daily in-

Table 1

Recommendations for iron and folic acid supplementation in women for prevention of anemia.

Target population	Nutrient	Product	Dose	Frequency	Start	Duration	Source
Women of reproductive age *	Iron	Iron sulfate	60mg	1x/week	3 months prior to conception	3 months	WHO [21]
	Folic acid **	Pteroylmono-glutamate acid	180µg ***	1x/day			IoM [24]
Pregnant women	Iron	Iron sulfate	60mg #	1x/day	20th week of pregnancy	Up to 3 months post-partum	WHO [21]
	Folic acid	Pteroylmono-glutamate acid	0.4mg ***		1st month of pregnancy ##	Until end of pregnancy	IoM [24]
Women after birth or miscarriage	Iron	Iron sulfate	60mg	1x/day	Immediately after birth	3 months	WHO [21]
	Folic acid	Pteroylmono-glutamate acid	0.4mg ***				

IoM: Institute of Medicine; WHO: World Health Organization.

* In population groups where the prevalence of anemia is greater than 20% among women of reproductive age, weekly supplementation should be considered as a strategy for preventing iron deficiency, improving pre-pregnancy iron reserves and improving folate status in some women. In the absence of anemia data for this group, the prevalence of anemia (> 40%) in other groups can be used as proxy: pregnant women or children aged < 5 years. In the absence of this data, criteria such as dietary and socio-economic patterns may be employed [21];

** Supplementation with folic acid in this group may not be required in two situations: within regions where food fortification has proven effective; and in regions endemic for malaria where anti-folate treatment is employed, since evidence suggests that supplementation with folic acid may reduce the efficacy of these drugs [21];

*** Diet + supplement;

Studies show that low daily doses of iron (30mg) during pregnancy improves maternal iron status and appears sufficient to protect the child from iron-deficiency anemia [10];

No collateral effects have been reported as a result of supplementation with folic acid from start of pregnancy.

take of folic acid [35]. It is noteworthy that neural tube defects constitute only one of the many problems linked to folic acid deficiency, since the vitamin is implicated in the etiology of other congenital anomalies such as Down's syndrome [36], cleft lip and palate defects [37].

- **Environment**

A low level of maternal education [38] and food shortage [39] have been identified as possible risk factors for neural tube defects, in addition to the use of medications which interfere with the metabolism of folic acid (anti-epileptics, aspirin, methotrexate, sulfasalazine etc.), hyperthermia, use of tobacco and excessive doses of vitamin A (> 15,000IU) during pregnancy [40]. The presence of abdominal obesity, diabetes mellitus and dyslipidemia may increase risk six-fold [41], while obesity alone almost doubles the risk of neural tube defects [42].

Technical advances in ultra-sonography providing more enhanced resolutions, together with the interruption of affected pregnancies, food fortification and intake of supplements containing folic acid, have substantially reduced the global prevalence of neural tube defects over the last three decades. These defects have an incidence of 1-2/1,000 births, varying with race and geographic region [40] both within and between countries. Worldwide, an estimated 240,000 cases of spina bifida and anencephaly, the most common forms of neural tube defects, occur annually [43].

The role of folic acid deficiency in pregnancy complications, such as higher rates of miscarriage and fetal malformation, was first studied in the 1960s [44], but the vitamin's role in preventing neural tube defects was only identified in the 1980s and 1990s [13]. Since this discovery, the consumption of folic acid-fortified foods and supplements have been recommended periconceptually in all American women of child-bearing age (0.4mg/day to prevent a first occurrence of neural tube defect; 4mg/day to prevent a recurrence), in addition to a healthy diet [45]. A recent systematic review indicated that periconceptional supplementation with folic acid alone had a protective effect of 72% against neural tube defects, but may influence multiple births [7].

In developing countries, the majority of pregnancies are unplanned [46], and this has been cited as the main stumbling block in implementing

recommendations to take folic acid for the prevention of neural tube defects. The disappointing results of public-health campaigns aimed at increasing the use of supplements [47] led to mandatory fortification of foods as an optimal solution to reach women in early stages of pregnancy. Over 50 countries, including Brazil, now fortify foods using folic acid [48], sparking worldwide debate on its benefits and risks.

In Brazil, congenital defects have a significant impact on infant morbidity and mortality, and constitute the second most frequent cause of infant death (13%) in the first year of life [49]. The cited study highlights the governmental and non-governmental actions in the country, evidencing the urgent need for a specific policy aimed at the support, treatment and prevention of congenital defects which, in the current context, hinge more on good planning than on technology.

The recommendations for folic acid supplementation in women for the prevention of neural tube defects are summarized in Table 2.

Use of synthetic folic acid

Supplementation

Ray et al. [50] reviewed the prevalence of folic acid supplementation in community programmes worldwide and found a variation of 0.9% (Southern Israel) to 49% (Vancouver, Canada) during the preconception period and 0.5% (Sicily, Italy) to 52% (Holland) during the periconceptional period. In the United Kingdom, the leading factor underlying low adherence was unintentional pregnancies, followed by the young age of mothers, low income and Hispanic ethnicity [51]. In Korea, a prevalence of periconceptional folic acid supplement use of 10.3% was observed, and was associated to a history of spontaneous miscarriage, planned pregnancy and knowledge of the benefits of folic acid [52]. Although the use of dietary supplements by pregnant and nursing American women has been little studied, data from the 1999-2000 National Health Nutrition Survey (NHANES) [53] showed that 52% of American adults took dietary supplements.

Knowledge on prevalence of folic acid supplement use during pregnancy in Brazil is scarce. A population-based transversal study (Pelotas, Rio Grande do Sul) reported a prevalence of folic acid supplement use during pregnancy of 31.8% versus 4.3% in the periconceptional period [54].

Many studies are available investigating the positive link between folic acid supplement use and improved nutritional status of the vitamin among women [55]. A random uncontrolled clinical trial (n = 40) was conducted comparing erythrocyte folic acid concentrations in women who were not pregnant (18-45 years) after supplementation with 1.1mg or 5mg/day of folic acid for 30 weeks. The results concluded that the 5mg group had significantly higher erythrocyte folate

Table 2

Recommendations for folic acid supplementation in women for prevention of neural tube defects.

Target population	Nutrient	Product	Dose *	Frequency	Start	Duration	Source
Women of reproductive age **	Folic acid	Pteroylmono-glutamate acid	0.4mg (if no previous pregnancy affected by neural tube defect);or 2.8mg 4.0mg (if a previous pregnancy was affected by neural tube defect)	1x/day 1x/week *** 1x/day	3 months prior to conception	6 months	WHO [21]

WHO: World Health Organization.

* Supplement, in addition to that provided by the diet;

** Because closure of the neural tube takes place by the 28th post-conception day, supplementation to prevent neural tube defects will only have an effect if started during the periconceptional period; however, this dose is still recommended by WHO specialists even after onset of pregnancy [3];

*** Weekly supplementation is an option only for women of reproductive age; after becoming pregnant, the WHO recommends daily supplementation as listed above [21].

concentrations than the 1.1mg group at study endpoint [56]. The authors adopted a cut-off point of 906nmol/L of erythrocyte folate as protective against neural tube defects, although failed to point out that the group supplemented with 1.1mg attained steady state erythrocyte folate concentrations (1,625 ± 339nmol/L) at levels 80% greater than the cut-off point. The cited study was funded by a pharmaceutical company.

Users of the supplement tend to take higher-than-recommended doses. A pharmacoepidemiological study in pregnant users of pre-natal primary care services in the city of Piracicaba, São Paulo, found that 47.9% of prescriptions were above the recommended daily dose [57].

Fortification

According to estimates by Bell & Oakley Jr. [58], 27% of the world population has access to flour fortified with iron and/or folic acid. Fortification with folic acid produced positive results in the United States [59], Canada [60] and Chile [61], reducing the frequency of neural tube defects. Other possible impacts of this policy are emerging, some potentially beneficial such as lower plasma homocysteine levels [62], and some harmful. Investigations carried out in the last decade have suggested that excessive intake of synthetic folic acid may promote the progression of undiagnosed neoplastic lesions, as seen with aberrant methylation of DNA in colorectal carcinogenesis [63], and has been linked to recurrent early pregnancy loss [64], vitamin B12 deficiency [65] and multiple births [66].

The Medical Research Council Vitamin Study (MRC), a random double-blind controlled clinical trial was a reference study for the establishment of the recommendation of 0.4mg/day of folic acid in women of reproductive age, leading to an estimated decrease in the incidence of neural tube defects of around 70% [13]. It is important to note however that the MRC sample comprised women who had previous pregnancies affected by neural tube defects and also that the placebo group was not "pure" (having taken iron and calcium pills). A study [67] assessing the relationship between folic acid supplementation and its sera levels (involving clinical trials which used folic acid doses > 1mg/day) and the effect of folic acid levels on the occurrence of neural tube defects (based on results from a cohort of 56,000 pregnant women) found that for a 0.4mg/day increase in folic acid intake, the reduction in risk for neural tube defects depended on the initial sera concentration of the vitamin, where the higher the initial concentrations, the lower the preventive effect. Moreover, for a given increase in folic acid intake, the sera concentrations of the vitamin increased more in older individuals than in younger ones [67]. Thus, it is questionable whether the recommended 0.4mg/day of folic acid for preventing neural tube defect applies to all population groups.

With regard to fortification, the quantity of folic acid (140µg/100g of flour) was determined with the intention of providing an additional average intake of 100µg/day, while preventing daily intake from exceeding 1 mg/day in American adults. Nevertheless, later studies showed that fortification in the United States was supplying more than twice the mandated amount [68]. Some months after fortification, reductions in folic acid deficiency were observed along with lower rates of hyperhomocysteinemia in the American general population, as well as a fall in the incidence of neural tube defects[69]. In parallel, the frequency of high blood folate concentrations (> 20ng/L) also rose among children and the elderly from 5% and 7% to 42% and 38%, respectively [70]. After a period of overexposure to folic acid however, the intake of the vitamin was found to dip in American women of reproductive age, supposedly due to the popularity of low carbohydrate diets with consequent reduced levels of fortification. This phenomenon may explain the decrease in sera and erythrocyte folate concentrations seen in the NHANES survey from 1999-2004 [71], suggesting that the effect of fortification on the risk of neural tube defects may be lower first assumed.

Studies on data from the Framingham Offspring Cohort showed that fortification more than doubled serum folate and increased erythrocyte folate by 30%, and led to a 50% reduction in the prevalence of high homocysteine. In Australia, a population-based study found a 38% increase in serum folate and a 21% decrease in average homocysteine concentration [72].

Fortification in Canada has led to an estimated reduction in prevalence of open neural tube defects of 50% [73] to 70% [74]. Molloy et al. [75] argued that increased levels of fortification in these areas are unlikely to further reduce the prevalence of neural tube defects, and it is therefore necessary to identify other modifiable risk factors such as inadequate vitamin B12 status.

The Brazilian government introduced mandatory fortification of wheat and maize flour with 150µg/100g in June 2004 [76]. The National Institute of Metrology, Normalization and Industrial Quality (INMETRO) analyzed six brands of corn flour (3 sourced from Paraná state, 2 from Rio de Janeiro and 1 from Minas Gerais) and all conformed to the minimum quantity required by law [77]. Soeiro et al. [78] assessed folic acid in samples of five brands of wheat flour and three brands of corn flour in the city of Campinas, São

Paulo: 12% (wheat) and 21% (corn) of the samples tested contained the quantity recommended by law, 60% (wheat) and 21% (corn) were low in folic acid, while 28% (wheat) and 58% (corn) exceeded mandated levels. Although these findings are important, there is currently a lack and need of national data quantifying folic acid in these flours and their derivatives.

To date, no population-based studies have been conducted assessing the impact of flour fortification with folic acid on the prevalence of neural tube defects in Brazil. A longitudinal study using data from the National System of Live Births (SINASC) from 2000 to 2006 (n = 161,341) carried out in Recife found no statistically significant difference between prevalence of neural tube defects during pre-fortification (0.75:1,000 live births) and post-fortification (0.51:1,000 live births) periods [79]. Preliminary data are available for other South American countries from the Latin-American Collaboration Study on Congenital Malformations (ECLAMC) for the period spanning from 1999 to 2001 [80]. Among countries included in the survey, only Chile, where fortification levels have been 220µg/100g of flour since 2000, showed a statistically significant reduction in rates of neural tube defects, although the authors did not rule out the possibility of "ecological fallacy".

The process of implementing fortification programmes in some countries, particularly in Europe, has been guided by comprehensive appraisal plans for monitoring the effectiveness and safety of the interventions. In Ireland for example, after accepting the recommendation of the National Committee on Folic Acid Food Fortification in 2006, the government charged a group of specialists to perform a preparatory study prior to implementing the programme for mandatory fortification of bread. Key indicators for assessing the future effects of higher folic acid intake by the population were studied: prevalence and incidence of pregnancies affected by neural tube defects; estimate of the degree of voluntary fortification with folic acid; blood folic acid status among subgroups of the population [81].

According to a guidelines published by the WHO [82], implementation of a food fortification programme entails the following steps: (1) determination of the nutritional status of the population with regard to the nutrient in question; (2) election of a suitable food vehicle; (3) establishment of the acceptability and stability of the fortified vehicle; (4) assessment of the bioavailability of the nutrient in the chosen vehicle; (5) conducting of a controlled field study and; (6) implementation of a regional or national fortification programme.

In Brazil's case, with regard to adherence to the steps outlined above, there have been insufficient studies assessing whether all age groups, or regions of the country have benefited from fortification (step 1). Table 3 contains a calculation of folic acid consumption based on estimates of food expenditure drawn from the *Brazilian Household Budget Survey* (HBS) [83], based on the assumption that fortification in Brazil takes place as stipulated by law (150µg/100g flour). Values approach those expected for the United States, of around 100µg/day from fortification, yet it is pertinent to ask: What is the distribution of the folic acid deficiency across different regions of Brazil? Which age/physiological groups benefit from fortification? Do these groups consume wheat or corn flour? Is any specific group at risk ?

In fact, the lack of nationally representative data on folic acid status is a problem common to other countries worldwide. A recent review[17] revealed that only ten countries (Costa Rica, France, Germany, Mexico, New Zealand, Norway, Switzerland, England, the United States and Venezuela) conduct surveys on folic acid status among the national population, although data generally relates to specific age or physiological groups thereby precluding extrapolation.

The decision to elect wheat and corn flour as the vehicles for folic acid in Brazil (step 2) was based on the premise that these foods are widely consumed by the Brazilian population. These flours however, constitute ingredients of processed foods such as cakes and biscuits (creams or otherwise) which are sources of nutrients known to be harmful to health, including simple carbohydrates and saturated and trans fats. Manufacturers of these products exploit the fortification factor as a selling point in their marketing strategy.

With regard to step 3, international studies have demonstrated that folic acid in flour was organoleptically well accepted, given that its stability was not significantly changed [84]. It was found that the addition of folic acid, as well as other nutrients, at different levels of fortification, had only a marginal effect [85].

In terms of the bioavailability of folic acid in vehicles chosen (step 4), amounts up to 0.5mg/kg of wheat or corn flour yielded bioavailability of over 85% [86].

Community surveys (step 5) should be conducted in order to assess potential risks and efficacy of fortification across different regions in Brazil. Despite the absence of these studies, the fortification programme went ahead in Brazil.

Table 3

Average folic acid (FA) intake in Brazil based on estimated food expenditure from the 2002-2003 *Brazilian Household Budget Survey* (HBS) [83].

Food item	FA or folate content (ug)/100g flour or natural source	All regions		North		Northeast	
		Daily dietary intake per capita (g)	Estimated FA or folate intake (µg)/ day	Daily dietary intake per capita (g)	Estimated FA or folate intake (µg)/ day	Daily dietary intake per capita (g)	Estimated FA or folate intake (µg)/ day
Fortified foods			128.07		85.27		128.19
Wheat flour	150	13.93	20.90	5.91	8.87	4.07	6.11
Corn cream sauce	150	0.50	0.75	0.17	0.25	1.55	2.33
Corn flakes	150	1.03	1.55	0.37	0.55	2.12	3.18
Corn flour	150	8.73	13.10	4.69	7.04	16.45	24.68
Pastry * (70% wheat flour)	150	9.17	13.76	5.98	8.97	9.02	13.53
Bread * (80% wheat flour)	150	44.49	66.74	34.56	51.84	43.44	65.16
Cakes * (50% wheat flour)	150	0.95	1.43	0.31	0.47	0.62	0.92
Biscuits * (50% wheat flour)	150	6.58	9.87	4.85	7.27	8.18	12.27
Natural sources			10.18		7.45		14.34
Boiled spinach	146	0.43	0.63	0.07	0.11	0.61	0.88
Cow's liver	253	1.17	2.96	1.49	3.76	1.54	3.90
Cooked beans	48	4.32	2.07	2.13	1.02	14.64	7.03
Raw beetroot	109	1.15	1.25	0.82	0.89	0.68	0.74
Boiled broccoli	108	0.30	0.32	0.02	0.02	0.01	0.01
Iceberg lettuce	29	1.76	0.51	1.72	0.50	1.15	0.33
Boiled cauliflower	44	0.48	0.21	0.07	0.03	0.07	0.03
Oranges	30	6.01	1.80	1.93	0.58	4.22	1.26
Boiled kale	93	0.35	0.33	0.50	0.47	0.10	0.09
Cucumber with skin	7	1.33	0.09	1.03	0.07	0.89	0.06
Fortified foods + natural sources			138.25		92.72		142.52

Food item	Southeast		South		Mid-west	
	Daily dietary intake per capita (g)	Estimated FA or folate intake (µg)/day	Daily dietary intake per capita (g)	Estimated FA or folate intake (µg)/day	Daily dietary intake per capita (g)	Estimated FA or folate intake (µg)/day
Fortified foods		123.89		184.50		81.76
Wheat flour	10.17	15.25	49.25	73.87	10.72	16.08
Corn cream sauce	0.12	0.17	0.02	0.02	0.04	0.05
Corn flakes	0.85	1.28	0.18	0.27	0.23	0.35
Corn flour	5.87	8.81	7.47	11.20	2.45	3.68
Pastry * (70% wheat flour)	9.67	14.51	10.83	16.25	6.75	10.12
Bread * (80% wheat flour)	48.65	72.98	46.72	70.08	29.54	44.31
Cakes * (50% wheat flour)	1.12	1.67	1.68	2.52	0.52	0.78
Biscuits * (50% wheat flour)	6.14	9.21	6.85	10.28	4.25	6.38

(continues)

Table 3 (continued)

Food item	Southeast		South		Mid-west	
	Daily dietary intake per capita (g)	Estimated FA or folate intake (µg)/day	Daily dietary intake per capita (g)	Estimated FA or folate intake (µg)/day	Daily dietary intake per capita (g)	Estimated FA or folate intake (µg)/day
Natural sources		8.97		8.60		7.21
Boiled spinach	0.35	0.50	0.64	0.93	0.14	0.20
Cow's liver	1.15	2.92	0.45	1.14	1.00	2.54
Cooked beans	0.10	0.05	0.08	0.04	0.15	0.07
Raw beetroot	1.21	1.32	1.63	1.78	1.95	2.13
Boiled broccoli	0.54	0.58	0.42	0.45	0.04	0.04
Iceberg lettuce	1.84	0.53	2.76	0.80	1.67	0.49
Boiled cauliflower	0.78	0.34	0.68	0.30	0.29	0.13
Oranges	7.98	2.39	6.82	2.04	4.02	1.21
Boiled kale	0.26	0.24	1.02	0.95	0.32	0.30
Cucumber with skin	1.27	0.09	2.37	0.17	1.50	0.10
Fortified foods + natural sources		132.86		193.10		88.97

* Values in parentheses represent estimated average percentage of wheat or corn flour contained in the food.

Adverse effects of synthetic folic acid

The debate over fortification with folic acid, despite its success in reducing rates of neural tube defects in some countries, has intensified in recent years in light of several fresh theories and findings [87,88].

A study on immune function in post-menopausal women found that activity of natural killer cells was influenced by intake of folic acid supplements ≥ 400µg/day. Natural killer activity is important in the defense against infection as well as in eliminating cancerous cells. This activity was reduced by 25% in women who consumed diets with adequate folate supplemented with folic acid. Moreover, unmetabolized folic acid was detected in the blood of 78% of women in this group [89].

Kelly et al. [90], in an uncontrolled, random clinical trial, found levels of unmetabolized serum folic acid post-prandially in volunteers (18-42 years) who consumed bread fortified with 266µg of folic acid/meal for five days. These authors pointed out that, depending on the levels of fortification, unmetabolized folic acid can persist in plasma for many years, particularly in individuals consuming large amounts of fortified foods. The authors also called for a review of the 1mg/day Tolerable Upper Level Intake (UL) of folic acid. Many women who adhere to the 400µg/day recommendation may present consistently high concentrations of unmetabolized folic acid in plasma. The saturation of dihydrofolate reductase (DHFR), an enzyme which slowly converts folic acid into tetrahydrofolate for use by the organism, partially explains the build-up of unmetabolized folic acid in plasma, implying that benefits of consuming high doses of folic acid are indeed limited [91].

Several mechanisms have been postulated as contributing to the risk of disease among individuals taking folic acid supplements and frequently manifesting unmetabolized folic acid in the bloodstream. One such effect is based on the fact that folic acid shares the same mechanisms of cell transport as MTHF in most tissues, meaning that high blood folic acid levels may compete for cellular transport with MTHF, needed for normal metabolic reactions. Studies have shown that gene expression of the cellular transporters decreases after incubation of human intestinal and renal cells with high concentrations of folic acid [92]. Another proposed mechanism is that high intracellular concentrations of folic acid may promote the synthesis and use of greater quantities of MTHF which, through its B12-dependent pathway for DNA synthesis, may deplete vitamin B12 needed for normal cognitive function [93].

A number of studies have shown a relationship between use of folic acid supplementation and higher risk of cancer. A prospective, nine-year study involving more than 11,000 individuals in Sweden, a country without mandatory fortification, showed a lower incidence of breast cancer in women with high folic acid intake [94]. Another study in a cohort of over 25,000 post-

highest quintile of folic acid intake, derived from both diet and supplements [95]. A prospective study in more than 1,000 men and women followed up for ten years after surgical resection for colorectal cancer found a 52% greater risk of recurrence of high-grade dysplastic adenomas in individuals taking folic acid supplements (10.9%) compared with those taking placebo (4.3%) [96]. Knowledge on the mechanisms by which folic acid stimulates the development of some types of cancer is explored in the process of devising anti-cancer drugs, which interfere in the functioning of enzymes metabolizing the vitamin [97].

Genetic damage in the presence of synthetic folic acid have also been a focus of investigations. A controlled, double-blind, random clinical trial in 56 healthy male and female volunteers (20-60 years) observed a significant decrease in the incorporation of uracil ($p < 0.05$) in a supplemented group (1.2 mg folic acid) compared to a placebo group (glucose). Incorporation of uracil was more sensitive to folic acid status in healthy volunteers than other biomarkers of DNA damage [98].

Through their metabolic interactions, folic acid and vitamin B12 protect the organism against anemia and cognitive decline. Vitamin B12 is needed for the functioning of methionine synthase, the enzyme responsible for remethylation of homocysteine to methionine and for converting N5-methylTHF to THF. In conditions of B12 deficiency, folic acid remains in N5-methylTHF form and N5, N10-methyleneTHF needed for DNA synthesis is not produced leading to erythrocyte macrocytosis. If the diet is supplemented with high doses of folic acid, macrocytosis secondary to vitamin B12 deficiency can be corrected since excess folic acid is converted into dihydrofolate (DHF) which is then converted into THF, restoring the process of normal DNA synthesis [99]. A study involving more than 1,500 elderly from the 1999-2002 NHANES survey found that low vitamin B12 status with normal folic acid status (20% of group) doubled anemia risk to 7%, while increasing dementia risk from 18% to 25%. In addition, the incidences of anemia and dementia in individuals with low B12 status yet high folic acid status due to supplements use (3.3 % of group), increased significantly to 15% and 45%, respectively[100].

These studies are summarized in Table 4.

Final considerations

The inclusion of family planning campaigns into the routine of public-health services could prevent the high number of cases of pregnancies in which the mother is unaware of conception, thus allowing commencement of supplementation prior to pregnancy and throughout maternity. This measure would not only prevent folic acid deficiency during the most critical period of embryogenesis but also avoid indiscriminate use of the supplement. Evidence has shown that, even in developed countries such as Japan, information on the role of periconceptional intake of folic acid is not sufficiently disseminated among young women [101]. Improvements in maternal health and the provision of quality health services in the area of human reproduction constitute essential actions to eliminate the multiple causes underlying infant mortality.

From a nutritional standpoint, since folate is mainly found in fresh fruit and leafy vegetables, individuals who are deficient in the vitamin due to under-consumption of these foods can also present deficiency in other important nutrients. Thus, the incentive to consume fruit, legumes and vegetables, besides having no adverse effects, can yield greater health benefits. All health professionals involved in the contraceptive care of women can contribute by propagating this information, placing emphasis on the protective effects of a healthy diet, particularly of a folate-rich diet.

Scientific evidence indicates that the strategy with the greatest impact in reducing the prevalence of maternal anemia and neural tube defects is supplementation aimed at target groups with close monitoring of treatment doses and duration. It is important to bear in mind that the benefits of fortification are clear, but that various adverse effects exist, and for every pregnancy with neural tube defect prevented, hundreds of people may be exposed to high levels of folic acid intake. A cautious approach to implementing universal fortification policies has been urged since 1995 [102]. Many countries have opted against mandatory fortification, primarily because additional health benefits are not yet proven in clinical trials, secondly due to the unknown risks, and thirdly out of respect for individuals' freedom of choice [103].

Table 4

Studies assessing adverse effects of excessive synthetic folic acid intake.

Reference	Place/Year	Study design	Characteristics of population (n/age/ physiological status)	Exposure (dose/ duration/period)	Results/Adverse effects
Troen et al. [89]	USA/2006	Transversal (subsample of population of a study in the greater Seattle recruited for an intervention trial)	Healthy premenopausal women (n = 105)	Dietary intake (measured by food frequency questionnaire) and number of supplement pills/ day, in the year preceding blood collection	Women with high folate intake (> 233µg/day) had lower cytotoxicity of natural killer (NK) cells if they also took folic acid supplements (> 400µg) (p = 0.02). NK cells are part of the specific immune response and can destroy a variety of cells infected by virus and tumor cells, protecting against cancer
Kelly et al. [90]	Ireland/1997	Uncontrolled, randomized, clinical trial	Healthy volunteers (5 men and 18 women), aged 18-42 years	Intake of bread fortified with 266µg folic acid/ meal, for 5 days	Presence of unmetabolized folic acid in plasma
Stolzenberg-Solomon et al. [95]	USA/2006	Cohort	25,400 women (aged 55-74 years) from Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial	Dietary intake (measured by food frequency questionnaire) and multivitamin in the year preceding blood collection	Adjusted RR were 1.19 (95%CI: 1.01; 1.41; p for trend = 0.04) for women reporting use of 400µg/day of folic acid supplements compared with those reporting no supplement use. Comparison between greatest and lowest quintile yielded an adjusted RR of 1.04 (95%CI: 0.83; 1.31; p for trend = 0.56) and 1.32 (95%CI: 1.04; 1.68; p for trend = 0.03) for intake of dietary folate and total folate, respectively
Cole et al. [96]	USA/2007	Controlled, double-blind, randomized, clinical trial	1,021 men and women (21-80 years) with recent history of colorectal adenomas and no previous intestinal carcinoma	Intervention group: 1mg/day of folic acid (n = 516), control group: placebo (n = 505) Both groups were randomized separately to receive aspirin (81 or 325mg/ day) or placebo	During the first 3 years, the incidence of at least 1 colorectal adenoma was 44.1% for the folic acid group (n = 221) and 42.4% for the placebo group (n = 206) (RR = 1.04; 95%CI: 0.90; 1.20; p = 0.58). The incidence of at least one advanced lesion was 11.4% for the folic acid group (n = 57) and 8.6% for the placebo group (n = 42) (RR = 1.32; 95%CI: 0. 90; 1.92; p = 0.15). In subsequent 5-year follow-up, the incidence of at least one colorectal adenoma was 41.9% for the folic acid group (n = 127) and 37.2% for the placebo group (n = 113) (RR = 1.13; 95%CI: 0.93; 1.37; p = 0.23); and the incidence of at least one advanced lesion was 11.6% for the folic acid group (n = 35) and 6.9% for the placebo group (n = 21) (RR = 1.67; 95%CI: 1.00; 2.80; p = 0.05). Folic acid was associated with higher risks of having 3 or more adenomas and non-colorectal cancers. After a 10-year follow-up, a 52% higher risk of recurrence of high-grade dysplastic adenomas was observed in those taking folic acid supplements (10.9%), compared to those taking placebo (4.3%) (RR = 2.52; 95%CI: 1.28; 4.98; p = 0.008)

(continues)

Table 4 (continued)

Reference	Place/Year	Study design	Characteristics of population (n/age/ physiological status)	Exposure (dose/ duration/period)	Results/Adverse effects
Basten et al. [98]	England/2006	Controlled, double-blind, randomized, clinical trial	Healthy male and female volunteers (n = 56), aged 20-60 years	Intervention group: 1.2mg folic acid, control group: glucose	A significant decrease in incorporation of uracil ($p < 0.05$) was observed in the supplemented group compared with the placebo group. Incorporation of uracil is more sensitive to folic acid status in healthy individuals than other biomarkers of DNA damage
Morris et al. [100]	USA/2007	Transversal population-based	1,459 elderly (≥ 60 years) participants in the National Health and Nutrition Examination Survey (NHANES)	Vitamin B12 status and sera methylmalonic acid levels (MMA) Low vitamin B12 status was defined as sera vitamin B12 concentrations < 148ρmol/L or sera MMA concentrations > 210nmol/L	In the group with low vitamin B12 status, sera folate > 59nmol/L (80^{th} percentile), in contrast to sera folate = 59 nmol/L, was associated with anemia (OR = 3.1; 95%CI 1.5; 6.6) and cognitive damage (OR = 2.6; 95%CI: 1.1; 6.1). In the group with normal B12 vitamin status, the OR for high versus normal sera folate for these outcomes were < 1.0 ($p_{interaction} < 0.05$), but significantly < 1.0 only for cognitive damage (0.4; 95%CI: 0.2; 0.9)

OR: odds ratio; RR: relative risk.

Resumo

A deficiência de ácido fólico tem sido associada ao risco de anemia e outros desfechos adversos na gravidez, como os defeitos no tubo neural. As recomendações atuais para prevenção de tais agravos são consideradas difíceis de se alcançar apenas por meio da dieta, e a suplementação com ácido fólico e a adição dessa vitamina a alimentos sob a forma de fortificação representam esforços centrais no controle da sua deficiência. É necessário, porém, conhecer as características da dieta habitual e o uso de suplementos entre mulheres em idade reprodutiva, com avaliação adequada de outros nutrientes da dieta. Ao lado dos efeitos benéficos observados em ensaios clínicos aleatórios controlados, riscos à saúde da população têm sido avaliados e largamente discutidos no meio científico: para que uma fração seja beneficiada pela fortificação, centenas de pessoas são expostas a quantidades de ácido fólico talvez elevadas e provavelmente incomuns, em quantidade e apresentação química, ao organismo humano.

Ácido Fólico; Ácidos Pteroilpoliglutâmicos; Epidemiologia Nutricional

Contributors

M. A. Cardoso planned and provided guidance on the organization of the study. L. C. Almeida carried out the literature search and produced the initial draft of the manuscript. L. C. Almeida and M. A. Cardoso reviewed the final version of the manuscript.

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