Original Communication

Efficacy of Adding Folic Acid to Foods

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Abstract: In the past, food fortification along with nutritional education and the decrease in *food costs* relative to income have proven successful in eliminating common nutritional deficiencies. These deficiencies such as goiter, rickets, beriberi, and pellagra have been replaced with an entirely new set of "emergent deficiencies" that were not previously considered a problem [e.g., folate and neural tube defects (NTDs)]. In addition, the different nutrition surveys in so-called affluent countries have identified "shortfalls" of nutrients specific to various age groups and/or physiological status. Complex, multiple-etiology diseases, such as atherosclerosis, diabetes, cancer, and obesity have emerged. Food fortification has proven an effective tool for tackling nutritional deficiencies in populations; but today a more reasonable approach is to use food fortification as a means to support but not replace dietary improvement strategies (i.e. nutritional education campaigns). Folic acid (FA) is a potential relevant factor in the prevention of a number of pathologies. The evidence linking FA to NTD prevention led to the introduction of public health strategies to increase folate intakes: pharmacological supplementation, mandatory or voluntary fortification of staple foods with FA, and the advice to increase the intake of folate-rich foods. It is quite contradictory to observe that, regardless of these findings, there is only limited information on food folate and FA content. Data in Food Composition Tables and Databases are scarce or incomplete. Fortification of staple foods with FA has added difficulty to this task. Globally, the decision to fortify products is left up to individual food manufacturers. Voluntary fortification is a common practice in many countries. Therefore, the "worldwide map of vitamin fortification" may be analyzed. It is important to examine if fortification today really answers to vitamin requirements at different ages and/or physiological states. The real impact of vitamin fortification on some key biomarkers is also discussed. An important question also to be addressed: how much is too much? It is becoming more evident that chronic excessive intakes may be harmful and a wide margin of safety seems to be a mandatory practice in dietary recommendations. Finally, the "risk/benefit" dilemma is also considered in the "new" FA-fortified world.

Key words: fortification, folic acid, folates, prevention, vitamins, risks

Introduction

Vitamins and minerals are essential for human physical and mental health and survival. Only very small quantities of these micronutrients are needed, but deficiencies can have disproportionately large, often life-threatening effects [1]. Over the last few years, interest in micronutrient deficiencies has increased greatly since evidence has accumulated that micronutrients malnutrition may contribute substantially to the global burden of disease, particularly in the developing world, due to reliance of communities on plant-based diets and low per capita consumption of meat and meat products [2]. However, while micro-

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nutrient deficiencies are certainly more frequent and severe among disadvantaged populations, they do also represent a public health problem in industrialized countries. In this sense, different nutrition surveys in so-called affluent countries have identified "shortfall" of nutrients specific to various age groups and/or physiological status [3–4]. The increased consumption in developed countries of highly-processed, energy-dense, but somewhat micronutrient-poor foods is likely to adversely affect global micronutrient intake and final nutritional status [5].

Solutions to control and prevent micronutrient scarcities are available and affordable. The best way to prevent micronutrient malnutrition is to implement programs designed to educate people to diversify their diets, since a "balanced" regime, based on a combination of plant and animal foods as advised by most dietary guidelines, should provide sufficient vitamins and minerals. Nevertheless, this is far from being achievable everywhere because it requires universal access to adequate food and appropriate dietary habits [6]. From this standpoint, fortifying commonly eaten foods with missing micronutrients has the dual advantage of being able to deliver nutrients to large segments of the population without requiring radical changes in food consumption patterns. In fact, food fortification has stood out among public health interventions as one of the most effective methods of preventing nutritional deficiencies for more than 80 years, as a means of restoring micronutrients lost by food processing, contributing significantly to the virtual elimination of goiter, rickets, beriberi, and pellagra in the Western world [7–8]. Nowadays, a more reasonable approach is to use food fortification as a mean to support but not replace dietary improvement strategies (i.e. nutritional education campaigns) [9].

Folate deficiencies and solutions

Iron, zinc, iodine, vitamin D, and vitamin A have represented the most chronic forms of micronutrient afflictions in developed countries, while the prevalence of another set of "emergent deficiencies" such as folate and neural tube defects is thought to be of significant importance at present [8]. Folate is the generic term encompassing the naturally occurring folates including folic acid (FA), which is also the synthetic form of folate added to foods as a fortificant and used in dietary supplements. Folate metabolism and bioavailability in humans is not completely understood. FA is an essential micronutrient involved in the prevention of macrocytic anemia, and acts as a cofactor in cellular development and homeostasis throughout all life stages [10]. It has been identified as a potential relevant factor in the prevention of: cardiovascular diseases, by lowering serum homocysteine levels [11–12]; certain type of cancers such as colorectal and colon [13–14]; neurocognitive decline in the elderly [15]; and congenital abnormalities affecting the development of the spinal cord and central nervous system, known as neural tube defects (NTDs) [16–17].

The highest reported intakes of folate occur in populations with the highest consumption of leafy vegetables, such as countries that follow a Mediterranean dietary pattern. However, taking into account many problems with accurate measurement of folate intake, comparisons among countries suggest that intakes vary greatly according to differences in dietary behaviors across different regions, ethnic groups, age, and socioeconomic circumstances [18–19]. As scientific evidence for folic acid new functions is still inconclusive [20–21], the only established recommendations at present are aimed at women of childbearing age for reducing their risk of having a NTD-affected pregnancy. In 1998, the US Food and Nutrition Board advised that all women capable of becoming pregnant should consume a daily dose of 400 μg of synthetic FA, either in the form of fortified foods or supplements, in addition to naturally occurring folates from food [22]. Similarly, European governments advise daily allowances or adequate intakes for folate, ranging from 200 to 400 g/day intake for the target population [23]. Particularly, in Spain, the recommended dietary intakes have been set for FA in 400 g/day for women of childbearing age, 600 g/day for the second half of pregnancy, and 500 g/day for women who are breastfeeding [24].

The previous statements have been the basis for the introduction of three potential strategies to improve folate status among target populations, especially in women capable of becoming pregnant: pharmacological supplementation, mandatory or voluntary fortification of staple foods with FA, and the advice to increase the intake of natural folate food sources. Several studies have shown a higher bioavailability in humans for FA [25] and the result of these findings was the establishment and use of the Dietary Folate Equivalent (DFE). The term DFE accounts for the higher bioavailability of the synthetic form vs. the natural folate vitamers, where DFE equals the micrograms of food folate plus 1.7 times the micrograms of added FA. Use of DFE was recommended by the US Food and Nutrition Board of the Institute of Medicine for planning and evaluating folate intakes [22].

Worldwide folic acid fortification

The main public health measure for primary prevention of NTDs in many countries is the recommendation of FA supplements taken periconceptionally amongst women who are planning a pregnancy. Increasing awareness and knowledge of the role of periconceptional folate in women of child-bearing age by educational campaigns is difficult, as women do not generally plan their pregnancies and those at higher risk of NTD-affected pregnancies (lower socio-economic and educational status) are not always concerned by health promotion messages and are also less likely to be supplement consumers [26]. Although not all cases of NTDs can be prevented by increasing the intake of FA due to a "floor effect" [27], studies have suggested that 50 % to 70 % of cases could be prevented by the appropriate consumption of FA before conception and during early pregnancy [27–28].

With regard to the advice to increase dietary intake of naturally-occurring folate, efforts such as health promotion campaigns to achieve dietary changes on a population basis have been proven to have limited success . Not only does it require a behavioral change, but variations also need to be accessible, affordable, and sustainable [30]. In addition, some countries have introduced mandatory fortification of a staple food and/or allowed manufacturers to voluntarily fortify certain foodstuffs .

Voluntary fortification requires women to know about and choose fortified foods, or that commonly and regularly eaten staple foods are fortified (e.g., milk) and affordable. In other words, most women must consume the fortified foods whether or not they are aware of its FA content. In addition, variations in FA amounts added by different producers to diverse foodstuffs [32], or higher than expected content "overages" [10, 33], add difficulty to monitoring folate intakes from these foods by the population.

Mandatory fortification seems to be the key to overcome many of the drawbacks described above. Most women will consume fortified food regardless of socio-economic disparities or family planning. In 1996, the Food and Drug Administration (FDA) established regulations requiring that by 1998 all standardized enriched cereal grain products sold in the US to include 140 μg FA/100 g, and to provide for the addition of FA to breakfast cereals, corn grits, infant formulas, medical foods, and foods for special dietary use to ensure the recommended daily dose of 400 μg of synthetic FA in women capable of becoming pregnant. Afterwards, about 53 countries, including most of those in the Americas, created regulations for mandatory fortification of wheat flour with FA, although many of these programs have not been fully implemented and the existence of regulations does not imply compliance [34]. Conversely, other countries are still reluctant to implement this population-wide strategy, as in the case of the European countries, which only allow addition of this vitamin on a voluntary basis, with the exception of Kyrgyzstan and Turkmenistan [35]. Worldwide, 540 million more people gained access to wheat flour fortified with iron, FA, or both in 2007, an 8 % increase from three years before. The number of women aged 15 to 60 years with access to fortified wheat flour increased by 167 million, and the number of births that potentially benefited from wheat flour fortification increased by 14 million. Despite these successes, more than two-thirds of the world's population, including millions of women of childbearing age, still lack access to fortified wheat flour and its benefits. Fortification standards and practices vary from country to country, as do the specifications for the type and quantity of the nutrients added or not [36]. Particularly, in 2009, bread fortification has been blocked for safety reasons in New Zealand and in the UK, where the recommendation of the Food Standards Agency (FSA) in 2007 was turned back by the Chief Medical Officer for further review of the evidence [37]. Recently, following updated guidelines published by the US Preventive Services Task Force supported the recommendations mentioned above and promoted the continuation of mandatory FA fortification programs [38].

Impact of folic acid fortification: advantages and disadvantages

Food fortification campaigns require careful planning, programming, communication, and evaluation. These include the establishment of estimated adequate intakes for many countries, with accompanying methods for designing and evaluating nutrient intakes for population groups. The Dietary Recommended Intakes for these countries now provide tolerable upper intake levels (UL) for nutrients, defined as the highest level of intake of a nutrient, above which an individual may be at risk of adverse effects from excessive intake, as do several other agencies such as the World Health Organization (WHO)/Food and Agriculture Organization (FAO). Internationally, the Codex Alimentarius of FAO and WHO [4] have established general principles for the addition of vitamins and minerals to foods. For example, the guidelines on food fortification with micronutrients published in 2006 [6], outline FAO/WHO initiatives for FA. In addition to demonstrating improvements in nutritional status, it is desirable to show that fortification programs improve the health and function of the populations to which they are provided [6].

During the last two decades, numerous studies have evaluated the possible health benefits of an increased intake of FA on human health, revealing that low folate intake, low folate status, or high plasma homocysteine concentrations are independent risk factors for a number of adverse health outcomes, among others: cardiovascular diseases, some cancers, cognitive decline in the elderly, adverse pregnancy outcomes, and congenital malformations. However, it has been only demonstrated that FA supplementation effectively lowers homocysteine concentration [39]. On the contrary, the only well-documented benefit emerging from randomized controlled trials, nonrandomized intervention trials, and observational studies including pre–post evaluation after mandatory fortification, is the risk reduction of NTDs [37, 40].

Because food fortification is a public health intervention that affects a large number of people who consume a particular food, possible adverse effects of FA have been hypothesized, including cancer and tumor promotion, epigenetic hypermethylation, interference with anti-folate treatment, miscarriages, and multiple births, besides the masking of vitamin B_{12} deficiency and potential progression of neuropathy. In this sense, it is possible to exceed the tolerable UL for synthetic FA (1000 g for adults aged ≥19 years) set by the US Institute of Medicine (IOM) [22]. Taking into consideration that multiple sources of FA exist, including supplements and enriched foods, fewer than 3 % of US adults exceeded this UL from 2003 through 2006, and almost all of these adults consumed an average of more than 400 g of FA per day from supplements. In general, fortification programs must by their very nature benefit the at-risk group, hopefully benefit the entire population, and not put any part of the population at risk. In other words, programs must conform to an axiom of medicine, "At best, do no harm". To our knowledge, no available evidence supported the conclusion that FA intake, through fortified foods, causes adverse effects [41–42].

Decrease of neural tube defects (NTDs) incidence

NTDs are congenital malformations of the brain and spinal cord due to the incomplete development of the neural tube approximately 28 days post-conception, comprising the second most common group of serious birth defects which include anencephaly, encephalocele, and spina bifida, among others [40].

Many studies showed that mandatory FA fortification of cereal products in US, Canada, Chile and Australia are associated with a significant improvement in population folate status and also with significant reductions in NTD prevalence [43–45]. The rate of NTDs in the US and Canada dropped by 25 % [46] and 46 % [43], respectively, after food fortification. Mills [47] showed that the mandatory FA fortification program has made a major impact on the risk of having a baby with a NTDs and has greatly reduced the burden of folate-deficiency anemia in the US population. Nevertheless, NTDs are still an important cause of mortality and morbidity globally, with a conservative estimated incidence of 4,300,000 new cases a year resulting in an estimated 41,000 deaths and 2.3 million disability-adjusted life years (DALYS) [48]. In this sense, although food fortification in the US increased the serum folate levels of women of childbearing age to an acceptable level, less than 10 % of these women reached a red blood cell folate level (906 nmol/L) considered to be highly protective [49].

In a recent report on folic acid-preventable spina bifida and anencephaly, Bell and Oakley estimated that 27 % of the world's population now has access to FA-fortified flour, but pointed out that after 18 years of knowledge and scientific evidence, only 10 % of preventable birth defects are being limited by current fortification programs, particularly in developing countries [50]. It should be mentioned that the mechanism of action for the effect of FA on prevention of birth defects is still unknown. One hypothesis is that its role in the synthesis of DNA precursors is critical for the rapid cell proliferation associated with neural tube closure. More recently, the effects of FA on epigenetic variables such as DNA methylation and histone methylation have received increased attention [51]. Subsequent studies have established genetic predispositions for NTDs in offspring in the form of gene polymorphisms for enzymes involved in folate-dependent homocysteine metabolism. These latter findings help to explain how the mother's and the unborn child's genotypes and some environmental factors (e.g., folate intake) can all influence the risk of NTDs [52].

FA likely has its beneficial effects through its active metabolites, but harmful effects could also occur via these metabolites or through another mechanism. When FA intakes exceed a specific physiologic threshold, the ability of intestinal enzymes to convert the vitamin to the reduced form is exceeded, resulting in unmetabolized FA in the blood. Obeid *et al.* [53] explained that it is possible that unmetabolized FA may accumulate with folate supplementation, particularly in individuals with common genetic variants associated with reduced activity of dihydrofolate reductase (which transforms FA to various active forms).

Consequently, safety concerns cannot be "professionally" used as an argument against fortification, although such concerns are urgently in need of more research. It should be noted that despite success in reducing NTD rates in countries through FA fortification of enriched cereal grains, racial/ethnic disparities in the prevalence of NTDs persist, so research of other non-folate risk factors that may contribute to the disparity of outcomes must continue in this area [52].

Cardiovascular diseases and homocysteine concentration

Cardiovascular diseases (CVD) are the leading cause of death in the world, accounting for 30.9 % of global mortality and 10.3 % of the global burden of disease. As early as 1969, the amino acid homocysteine was hypothesized to affect atherosclerotic processes. Since that time, substantial evidence has accumulated linking high homocysteine levels in plasma and serum to the risk of CVD [54].

Folate and cyanocobalamin (vitamin B_{12}) are important regulators of the metabolism of homocysteine in the body, and many studies have shown an inverse relationship between these factors and homocysteine serum concentrations. Thus, low serum folate and elevated serum homocysteine levels have emerged as risk factors for coronary heart disease. However, the relationship between folate and homocysteine is complex, partially because of the many forms of FA, the interaction with B_{12} in the so-called "folate trap," and the previously mentioned genetic differences in the alleles for enzymes controlling FA conversion to its various active forms [55]. After mandatory FA fortification, plasma homocysteine concentrations have been assessed in relatively large population samples, which has resulted in the prevalence of hyperhomocysteinemia being markedly reduced [39]. Nevertheless, many randomized trials with FA for secondary prevention of cardiovascular disease have been performed with non-conclusive contradictory results. During the FA post-fortification era, the entire distribution of serum and red blood cell folate concentrations has shifted to states of sufficiency and even to excess in some populations such as the US [56]. The previously cited 2010 analysis of the data from the National Health and Nutrition Examination Surveys (NHANES) shows that only 2.7 % of the population had daily intakes above the UL of 1,000 g FA/day. Consequently, these researchers gave some assurances about the right level of FA for fortification since it is unlikely that US adults, who consume fortified foods and supplements averaging up to 400 g FA/day, would exceed the UL for FA. On the other hand, Yeung *et al.* [57] suggest that the majority of usual daily intakes in US children exceeded their age-specific UL by consumption of different sources of FA. Bazzano [58] explained that although the potential for prevention of atherosclerotic cardiovascular disease in adults through FA supplementation has not yet been substantiated, FA supplementation prenatally may reduce congenital heart defects in offspring. A meta-analysis [59] showed that even a 10 % benefit in reducing CVD could be ruled out with 84 % power, suggesting that longer term exposure might not be beneficial in preventing stroke [60]. Other studies concluded that although folate concentrations enlarge linearly with increasing doses of FA, the homocysteine concentration reaches a plateau when intakes of FA are increased. This latter feature allows for the conduct of dose-finding studies to find the lowest possible dose of FA for a maximal homocysteine-lowering effect [61]. Although these achievements may raise doubts about the efficacy of mandatory FA fortification, the evidence that FA lowers fasting concentrations of total homocysteine was welcomed as a justification to increase the intake of FA in the general population due to the greatest benefits from fortification predicted in myocardial infarction prevention [61–62].

Masking of anemia induced by vitamin B_{12} deficiency

The most widespread and well-known potential adverse effect of high FA is the masking of vitamin B_{12} deficiency, mainly in the elderly. B_{12} deficiency has been linked to poor pregnancy outcomes and increased risk of NTDs, delayed child development, abnormal cognitive function and depression, anemia, and elevated plasma homocysteine concentrations [63]. Megaloblastic (abnormally large red blood cell) anemia caused by B_{12} deficiency is clinically identical to megaloblastic anemia caused by FA. So, diagnosis of B_{12} scarcity may be delayed in individuals with increased FA intake. In addition, the neurological complications of B_{12} deficiency do not respond to FA, hence masking B_{12} megaloblastic anemia with FA may result in a delayed diagnosis and treatment of

B_{12} deficit, a condition that has been proven to cause irreversible neurological damage [64]. In this sense, the values for tolerable upper daily intake limits were established by taking into consideration only data related to the masking of B_{12} deficiency [65]. Based on these facts, different initiatives need to be considered before implementing mandatory B_{12} fortification of flour along with FA [66].

Cancer

The other major unresolved concern regarding fortification is its "dual" effect on cancer. Epidemiological studies have provided evidence that high folate intake may be associated with decreased risk of cancers of the colon and rectum, pancreas, and esophagus [67]. Of further and more recent concern is the association of FA with a potential increase in the risk of cancer, particularly colorectal [68]. FA has a role as a cofactor in nucleotide synthesis and its availability can promote proliferation of rapidly dividing malignant cells. Observations from both animal and human studies have suggested the possibility of a "dual effect" of this vitamin in cancer development, depending on the timing and dose of the intervention [68]. High intakes may suppress development of early lesions in normal tissues, probably by maintaining genetic stability, but may in turn increase the progression of neoplastic cells. This complex relationship that was referred to as the "double-edged sword" by Kim [69] is exerting a necessary halt/delay in the widespread FA fortification in many countries, while the safety of this policy is ensured for all population groups [70]. Moreover, adequacy of other vitamin statuses such as B_2, B_6, and B_{12} has to be considered, as they are clearly interrelated and serve as cofactors for crucial enzymes governing one-carbon metabolism. From this point, Stevens *et al.* [71] predicted that the chemical form of folate more beneficially influences colorectal cancer risk, since synthetic FA used in fortification and supplements can overwhelm these enzymes, resulting in circulating, unmetabolized FA.

The future of folic acid fortification

At present it seems clear that "optimal nutrition" remains a moving target in the food fortification field: in order to appropriately design a fortification program, the nutritional status of many micronutrients must be assessed. Furthermore, as nutrigenomic studies identify individuals with higher, or at least different nutrient requirements, nutrient fortification may help optimize an individual's nutritional needs. The scientific evidence base continues to evolve, and discovery of new compounds with health-promoting effects will call for new strategies involving both nutrition guidance and fortification with a focus on benefit-risk assessment [6].

Within this context, the increasing significance of folate in health or in disease, and the fact that the average daily intake of folate is somewhat below the recommendations for most groups (children, adolescent, fertile women, etc.), which may cause a worldwide health problem due to folate deficiency or insufficient intakes for newly emerging roles of the vitamin, emphasizes the need for a critical evaluation of all types of dietary folate sources to give a more reliable assessment of folate content in foods and commercial products [18–19,72]. Monitoring and surveillance are an essential part of the fortification program to ensure that the amount of FA in fortified products is within the range required by the regulation. However, it is quite contradictory to observe that, regardless of these findings, there is only limited information on food folate and FA content or about the significant and increasing number of FA-fortified products from different food groups and aimed at a wide variety of populations. In order to overcome these difficulties, the European Food Information Resource Network of Excellence (EuroFIR) project is making a great contribution in unifying and harmonizing Food Composition Databases across Europe .

In addition, adequate serving/portion size is still an untargeted issue for fortification of staples. Recommended and/or standard serving sizes are the basis for intake assessments, but it should be taken into account that they will vary depending on the population-age-gender group or physiological state. Moreover, FA contents/levels in fortified food labels do not always match label information. Different studies, including those from our research group, found overages in fortified products, since manufacturers may add higher quantities of this nutrient to ensure its presence at declared levels throughout shelf life [10,33].

Further studies comprising the monitoring of population intakes in countries where FA fortification is implemented at different levels, will grant greater insight into improving the diverse policy positions on mandatory FA fortification present in these countries, which reflect different interpretations of the potential risks and benefits [68]. Studies from Vergahen *et al.* [74] show a framework that allows quantitative comparison of human health risks and benefits of FA on foods based on a common scale of measurement.

These authors applied the novel tool BRAFO for benefit–risk assessment to case studies on dietary interventions. Accordingly, they establish an important question: "is the benefit to the relatively few mothers and children sufficient justification for exposing the entire population to an increased intake of FA?" The answer to this question remains uncertain because many issues remain unresolved regarding the benefits and the risks of FA fortification. For all these reasons, European authorities are still reluctant to implement mandatory FA fortification [75] or to consider other strategies to supply these necessities, such as through biofortification [76].

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