



American University of Armenia

Հայաստանի Ամերիկյան Համալսարան

A US-accredited institution affiliated with the University of California.

School of Public Health

**FORTIFICATION OF WHEAT FLOUR WITH FOLIC ACID AND IRON
BENEFITS AND RISKS**

A Literature Review

By Ani Movsisyan, MPH, MSc, PhD (c)

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LIST OF ABBREVIATIONS

CDC – Centers of Disease Control and Prevention
CI – Confidence Interval
CRC – Colorectal Cancer
CVD – Cardiovascular Disease
DFI – Dietary Folate Equivalents
FFI – Food Fortification Initiative
FSANZ – Food Standards Australia New Zealand
GAIN – Global Alliance for Improved Nutrition
IRR – Incidence Rate Ratio
NTD – Neural tube defect
RCT – Randomized Controlled Trial
RDA – Recommended Dietary Allowance
RR – Relative Risk
SACN – Scientific Advisory Committee on Nutrition
SMD – Standardized Mean Difference
WHO – World Health Organization
WMD – Weighted Mean Difference

EXECUTIVE SUMMARY

Food fortification – the addition of micronutrients to a processed food in order to improve its nutritional quality has gained significant importance in the recent decades as a cost-effective and easy to implement intervention to address micronutrient deficiencies in the populations. At present, 76 countries worldwide have legislations that mandate wheat flour fortification with at least folic acid and iron. Along these developments, several concerns have been raised regarding the potential adverse effects of food fortification programs. This paper aims to summarize findings from the extant scientific literature on the potential benefits and risks of food fortification programs, specifically focusing on wheat flour fortification with folic acid and iron. For these purposes, this paper conducted a comprehensive search in scientific databases and included peer-reviewed investigations on the effectiveness of flour fortification/supplementation programs with a major focus on the evidence from systematic reviews and meta-analyses. This literature review does *not* include assessment reports or publications funded by international donor organizations.

With regards to *the **benefits of flour fortification programs with folic acid***, the current scientific evidence demonstrates that:

- Folic acid fortification has significantly reduced the prevalence of neural tube defects (NTDs) in all countries, where it has been implemented.
- Folic acid fortification may reduce the incidence of stroke, however further research is needed to confirm this relationship.

With regards to the *potential **risks of flour fortification programs with folic acid***, this paper found that:

- Folic acid supplementation does not significantly increase the risk of total or site-specific cancer incidence. This implies that folic acid fortification programs do not pose a risk of total or site-specific cancer incidence, given they involve doses of folic acid that are, on average, an order of magnitude smaller than those used in the folic acid supplementation interventions. Furthermore, a recent investigation suggests that folic acid fortification might protect children aged 0 to 4 years from Wilm's tumor.
- The concerns on the risk of masking vitamin B₁₂ deficiency and therefore promoting development of neuropathy associated with vitamin B₁₂ deficiency, as well as the development of cognitive impairment associated with high levels of serum folate in the elderly is not confirmed by experimental evidence. This is also the case with the concern over the increased risk of twinning associated with high serum levels, which might be the result of using assisted technologies instead.
- Recent evidence suggests that because of differences in the processing of dietary folic acid and natural folate (the form found in green vegetables) by body, folic acid fortification might result in the accumulation of unmetabolized folic acid in the organism, the health risks of which are yet unknown. An alternative is to use natural forms of folate in mandatory folic acid fortification programs, which are currently licensed for use.

As for the ***benefits of flour fortification programs with iron***, the current state of the evidence suggests that:

- Iron fortified foods have the potential to positively affect hematologic outcomes of women and children, including the levels of hemoglobin and serum ferritin, and the risk of anemia and iron deficiency.
- The current practices of wheat flour fortification with iron worldwide are inadequate to yield expected beneficial outcomes, because they use inappropriate iron

fortificants that have reduced bioavailability, however are less expensive. Moreover, most of the programs use iron fortification levels that are too low in relation to the wheat flour fortification consumption patterns of the populations, which is another factor that might reduce their effect.

Finally, the main **risks of flour fortification programs with iron** are iron overload and infections. Both of these potential risks, however, are mostly associated with iron supplementation programs, that use higher doses of iron, and there is no consistent evidence suggesting real adverse effects as a result of iron fortification programs.

To sum up, the extant scientific literature suggests that mandatory wheat flour fortification with folic acid and iron is a safe and cost-effective intervention with a potential to have significant impact on the health of the population. However, in order to prove beneficial, fortification programs should be well monitored, updated according to emerging evidence on its potential adverse effects, and implemented in light of the key factors. Those include:

- Nutritional needs and deficiencies of the population
- The consumption profile of “fortifiable” flour
- Sensory and physical effects of the fortificants on flour
- Bioavailability of flour fortificants
- The processing of the fortificants by body
- Fortification of other food vehicles
- Consumption of vitamin and mineral supplements of the population and the specific subgroups
- Costs and benefits

INTRODUCTION

Food fortification is regarded as one of the safest and most cost-effective strategies to combat micronutrient deficiencies worldwide [1], which account for 7.3% of the global burden of disease, with iron and vitamin A deficiencies included in the 15 leading causes of global disease burden [2]. The populations most affected by micronutrient deficiencies are pregnant and lactating women and young children, given their increased demands [3]. According to the World Health Organization (WHO) estimates [4], every second pregnant woman and 40% of preschool children in the developing countries are anemic. The accumulated evidence on the health consequences of micronutrient deficiencies urged the international community to recognize their public health importance, resulting in a number of action plans discussed in international fora, as well as the launch of public-private coalitions such as the Global Alliance for Improved Nutrition (GAIN) to address the main micronutrient deficiencies.

In contrast to food *supplementation*, which relates to the provision of relatively large doses of micronutrients, in the form of pills, capsules or syrups, and is expected to provide the fastest improvements in the micronutrient status of targeted populations, food *fortification* refers to the addition of micronutrients to processed foods and tends to have less immediate, however a much wider and sustained impact [1]. WHO categorizes food fortification strategies into three groups: *mass*, *targeted* and *market driven* [1]. Mass fortification involves foods that are widely consumed, such as wheat, salt, oil, etc.; targeted fortifications are designed for specific population subgroups such as infant complementary foods; and market driven fortification involves manufacturers voluntarily fortifying foods of specific brands. In contrast to market-driven fortification, which is always *voluntary*, mass fortification is almost always *mandatory*, while targeted fortification can be either mandatory or voluntary. Among the highlighted issues of voluntary food fortification are the difficulties in knowing whether a particular food over time is consistently fortified with the same amount of a micronutrient, which challenges the monitoring of the overall intake of a micronutrient from consumption of voluntarily fortified foods in the general population, and the potential for exposure to higher intakes of a micronutrient for those who might consume large quantities of a specific fortified food [5]. Mandatory food fortification strategies can overcome these challenges, as well as address the socioeconomic disparities associated with voluntary programs [5].

The practice of food fortification, specifically vitamin A-fortified milk and iron and B complex flour began in many developed countries in the 1930s, while salt-iodization had already been introduced in Switzerland and the United States (US) in the early 20th century, and vitamin A-fortified margarine in Denmark – as early as 1918 [1, 6]. Food fortification programs are also being considered in many low- and middle-income countries. At present, there are 82 countries with a legislation to mandate fortification of industrially milled wheat flour with vitamins and minerals (see Figure 1). All these 82 countries have legislations for mandatory fortification of wheat flour with at least iron and folic acid except for Australia, which does not include iron, and Congo, Nigeria, Venezuela, the United Kingdom (UK), and the Philippines, which do not include folic acid [7]. With the support provided by GAIN, mandatory flour fortification with iron and folic acid has been recently introduced in Uzbekistan [8]. Under the national food subsidy program, all wheat flour is fortified with ferrous sulphate and folic acid for baladi bread – the traditional bread in Egypt that is estimated to reach 50 million Egyptians on a daily basis [9]. Finally, in 2009, Kyrgyzstan passed the law “On the Enrichment of Bread Flour” that supports a phased transition of all mills to mandatory flour fortification [10].

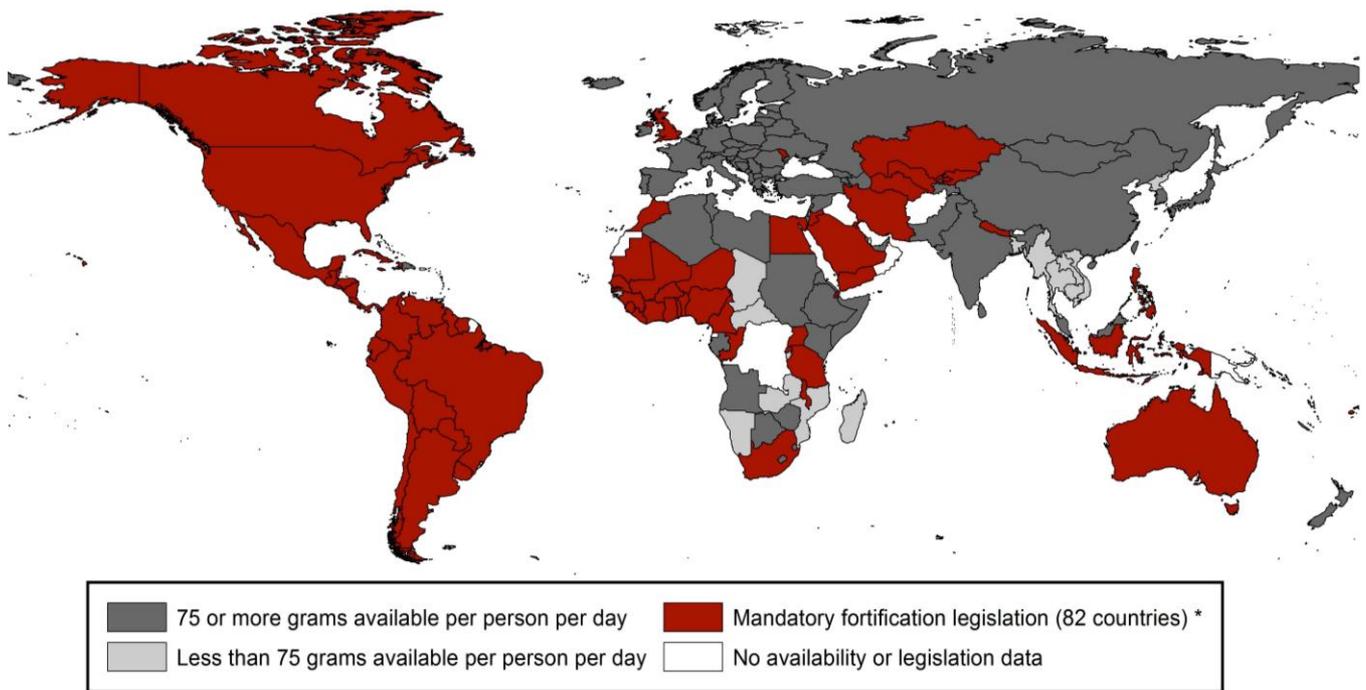


Figure 1. Wheat availability and fortification legislation [7]

Notes: *Legislation has the effect of mandating grain fortification with at least iron or folic acid. This does not reflect how much grain is available in that country.

Grain availability data from the Food and Agricultural Organization (2011).

Legislation status from the Food Fortification Initiative (www.FFInetwork.org).

Notwithstanding these developments and the efforts of the international partnerships, including the Food Fortification Initiative (FFI) that promote implementation and uptake of food fortification practices globally through provision of advocacy support and technical assistance, the efficiency of these practices have been questioned, in part because of concerns for their possible adverse health effects. By way of illustration, wheat flour fortification with iron was suspended in Denmark and Sweden, because of uncertainty of its effects and concerns for iron overload in the population [11, 12]. Furthermore, although “The Bread of Flour Regulations 1998” require all wheat flour to be fortified with iron, thiamine (vitamin B₁), nicotinic acid or nicotinamide and calcium carbonate in the UK [13], the mandatory fortification of flour with folic acid is currently being considered by the UK health ministers, following the recommendations by Scientific Advisory Committee on Nutrition (SACN). The latter recommends mandatory fortification of folic acid if it is accompanied by action to restrict voluntary fortification of foods with folic acid, measures to monitor emerging evidence on its adverse effects, and guidance on supplement use for particular population groups: specifically those aged over 50 years should not take supplements containing more than 200 µg/day folic acid a day because of the enhanced risks for colorectal adenomas/cancer after this age [14].

Because of the foregoing concerns and contentions, this paper aims to examine the latest scientific evidence on the benefits and risks of wheat flour fortification with folic acid and iron, and summarize the revised and the most recent recommendations for their practice. For these purposes this paper conducted a comprehensive literature search in scientific databases including MEDLINE and EMBASE. Additionally, this paper used references of identified relevant publications to further expand the scope of the literature review. This review does not include reports funded by international donor organisations, and summarizes literature on the effectiveness of fortification interventions retrieved from international scientific journals. Commensurate with the principles of evidence-based practice, and in contrast to focusing only on the evidence from individual trials, this paper

attaches more weight to the evidence from systematic reviews and meta-analyses that effectively synthesize results from several studies and trials.

Most importantly, and as highlighted in WHO recommendations, decisions about flour fortification should be based on considerations of key factors: nutritional needs and deficiencies of the population, the consumption profile of “fortifiable” flour, sensory and physical effects of the fortificant nutrients on flour, their bioavailability, fortification of other food vehicles, population consumption of vitamin and mineral supplements and costs [15].

FORTIFICATION OF WHEAT FLOUR WITH FOLIC ACID

Folate, folate deficiency and dietary folate equivalents (DFE¹)

Folate (vitamin B₉) has a key role in the synthesis and methylation of nucleotides that intervene in cell multiplication and tissue growth. Its role in protein synthesis is interrelated with that of vitamin B₁₂, and the combination of severe folate deficiency with vitamin B₁₂ deficiency can result in megaloblastic anemia (see Table 1) [1]. Folate deficiency defined as <7nmol/l (~3 µg/l) of serum folate or <315 nmol/l (~140 µg/l) of erythrocytes (red blood cell) folate (the latter serves as a long-term indicator of folate deficiency) [16] has shown to be associated with an increased risk of giving birth to infants with neural tube defects and possibly other birth defects [17, 18], and with a higher risk of cardiovascular disease and impaired cognitive function through its effect on plasma homocysteine levels [19, 20].

Folate deficiency is more prevalent in populations with high intake of refined cereals (which are low in folate) and low intake of leafy greens, legumes, fruits, yeast and liver (which are high in folate). Because of this, it is possible that populations in certain developing countries have higher folate levels than those in industrialized countries [1]. The recommended dietary allowance (RDA) for all males and nonpregnant females 14 years of age and older is 400 µg DFE/day, while for pregnant and lactating women, the RDA increases to 600 and 500 µg DFE/day, respectively [16].

Table 1. Folate deficiency: prevalence, risks factors and health consequences (data taken from WHO guidelines [1])

Nutrient	Prevalence of deficiency	Risk factors	Health consequences
Folate	Insufficient data	<ul style="list-style-type: none"> - Low intakes of legumes, vegetables, fruits, dairy products - Malabsorption and intestinal defects parasites infections (e.g. Giardia Lamblia) - Genetic disorder of folic acid metabolism 	Megaloblastic anemia. <i>Risk for:</i> <ul style="list-style-type: none"> - Neural tube defects and other birth defects - Elevated plasma homocysteine - Heart disease and stroke - Impaired cognitive function - Depression

¹ Dietary folate equivalents (DFE) are units that account for the higher bioavailability of synthetic folic acid as compared to naturally occurring food folate

Evidence of the effectiveness of folic acid supplementation and fortification programs

Neural tube defects (NTDs)

Recommendations for pregnant women to take a daily periconceptional supplement that contains 400 µg folic acid beginning at least 1 month before conception through early pregnancy is a well-established practice to prevent NTDs in many developed countries, guided by solid evidence of its protective effects [21]. Nevertheless, evidence from 18 European countries with periconceptional folic acid supplementation policies, however without mandatory folic acid fortification programs demonstrates that reliance on supplementation recommendations alone is insufficient to translate the known protective effects of folic acid into population-wide reductions in the prevalence of NTDs [22]. Specifically, data from the European network of population-based registries for congenital anomalies (EUROCAT; excluding UK and Ireland) showed that in countries that by 1999 had endorsed a policy for supplementation of folic acid or dietary intake of folate to increase folate status among pregnant women, there was no decline in the rates of NTDs from 1980 to 2002 (2% reduction; 95% CI: from 28% reduction to 32% increase) compared to countries with no such policy in place (8% reduction; 95% CI: from 26% reduction to 16% increase). A decline of 30% in the prevalence of NTDs was calculated in the UK and Ireland for this period, however, authors were not able to distinguish this from the strong decline observed prior to the policy endorsement [22]. Although this study did not directly assess the levels of uptake of folic acid supplements by pregnant women, one of the potential factors that might account for the failure of these interventions is the high rate of unplanned pregnancies in these countries. Therefore, the authors draw on folic acid fortification strategies as more effective alternatives.

Flour fortification with folic acid was mandated in the United States and Canada in the late 1990s [5], followed by a number of countries in South America in the early 2000s [23]. A recent systematic review that aimed to study the impact of folic acid fortification of wheat flour (i.e. national folic acid fortification programs) on the prevalence of NTDs in different countries found that fortification of flour with folic acid has significantly reduced the prevalence rates of NTDs in all countries that reported these data [24]. This review included 27 studies from 9 countries of changes in the prevalence rates of NTDs between pre-fortification and post-fortification periods. The included countries were: the USA, Canada, Argentina, Chile, Costa Rica, South Africa, Brazil, Jordan and Iran. The largest drops in the NTD prevalence were observed in Costa Rica (58%), Argentina (49.7%), and Canada (49%), while the largest drops in spina bifida – in Costa Rica (61%), Canada (55%) and Chile (55%). For anencephaly, the greatest reductions were observed for Costa Rica (68%), the province of Ontario in Canada (58%), Argentine (57%) and Chile (50%). The authors also conducted dose-response analysis for Chile, and found that the highest prevalence of NTDs was observed when wheat flour folic acid content was at its lowest (median = 1.1 mg/kg), and the lowest prevalence was observed when the median folic acid reached 1.5 mg/kg. It is however worth noting, that the *uncontrolled* design of the included studies, as well as the large variability of the data sources and failure to account for the stability of wheat flour-based food consumption patterns in these countries limit conclusions for causal relationships.

These results are confirmed by another recent systematic review that aimed to assess the effectiveness of food fortification with single and multiple micronutrients compared with no fortification on the health and nutrition of women and children [6]. With regards to folate fortification, a total of 31 before-after mass fortification studies were identified with flour as the food vehicle and fortification levels varying from 40 µg/100g to 500 µg/100g. Results showed that folate fortification had a significant impact in reducing NTDs (RR: 0.57; 95%

CI: 0.45 to 0.73), spina bifida (RR: 0.64; 95% CI: 0.57 to 0.71) and anencephaly (RR: 0.80; 95% CI: 0.73 to 0.87). However, no significant effect was observed on red blood cell folate levels or serum folate concentrations. Subgroup analysis revealed that use of different folate concentrations (40 µg/100g or more than 100 µg/100g) had consistent significant effect in reducing NTDs and spina bifida, while only 40 µg/100g folate fortification was effective in reducing the incidence of anencephaly [6].

Cardiovascular Disease (CVD)

Because of folate's potential effects on plasma homocysteine levels, and evidence suggesting that elevated plasma homocysteine levels are in turn associated with higher risks of CVD [19, 20], there have been scientific debates whether folic acid supplementation (as homocysteine lowering interventions) or fortification strategies are effective in reducing CVD risks. This question was brought to the front by a quasi-experimental study demonstrating improvements in stroke mortality after folic acid fortification in the US and Canada but not in England and Wales, where folic acid fortification is not required [25]. Specifically, the authors found that decline in stroke mortality observed in the US between 1990 and 1997 (pre-fortification period) accelerated in 1998 to 2002 (post-fortification period) with an overall change from -0.03% to -2.9% per year. The fall in stroke mortality in Canada averaged to -1.0% and accelerated to -5.4% per year in 1998 to 2002 (post-fortification period). In contrast, the decline in stroke mortality did not demonstrate significant changes between 1990 and 2002 in England and Wales. In addition, authors conducted sensitivity analysis for 4 main risk factors of stroke mortality (current cigarette smoking, hypertension, diabetes, and total serum cholesterol concentration of more than 239 mg/dL) and found that changes in these factors were not likely to account for the reduced number of stroke-related deaths [25]. The major drawback of this evaluation, however, was that the authors did not account for the changes in the stroke incidence in the US and Canada during this period, and it might be that the decline in stroke mortality was a result of a reduced case-fatality rate instead.

Three meta-analyses were conducted after the abovementioned study that studied the effects of lowering homocysteine levels with B vitamins (including folic acid) on CVD outcomes [26-28]. Two of these meta-analyses did not find any significant effect of folic acid supplementation on CVD outcomes or cancer risk, while the third study found a significant effect only when the supplementation was given in regions without folate fortification.

The first meta-analysis included 8 randomized, placebo-controlled studies of folic acid supplementation involving 37,485 individuals at increased risk of CVD [26]. Even though, folic acid allocation yielded 25% reduction in homocysteine levels, during a median five-year follow-up period, folic acid supplementation had no significant effect on any of the cardiovascular outcomes. Authors neither found any significant effect for overall cancer incidence, cancer mortality or all-cause mortality within 5 years.

The second meta-analysis is a recent Cochrane review that aimed to determine whether homocysteine-lowering interventions in the form of vitamins B₆, B₉, and B₁₂ supplements were effective in preventing cardiovascular events, as well as all-cause mortality when delivered to patients with and without pre-existing CVDs [27]. This review included 12 randomized controlled trials (RCTs; involving 47,429 individuals) from countries with and without mandatory fortification of foods. Authors found that homocysteine-lowering interventions had no significant effect on non-fatal or fatal myocardial infarction, stroke, and death from any cause or risk of cancer in comparison with placebo (the follow-up period for cancer incidence ranged from 3.4 to 7 years). Evidence for all these outcomes were given "high" quality scores.

Finally, the third meta-analysis aimed to investigate the potential effect of folate fortification on folic acid-based homocysteine-lowering intervention and stroke risk by stratifying previous RCTs according to the folate fortification status of the country or the region [28]. The review included 14 RCTs involving 39,420 patients, which were stratified into subgroups with folate fortification, without folate fortification and partial folate fortification. Authors found significant difference in the risk of stroke between the subgroups with folate fortification and without folate fortification ($p < 0.05$). The RR for stroke was 0.88 (95% CI: 0.77 to 1.00) in the subgroup without folate fortification, 0.91 (95% CI: 0.82 to 1.01) in the subgroup with partial folate fortification and 0.94 (95% CI: 0.58 to 1.54) in the subgroup with folate fortification. These findings imply that stroke reduction as a result of folic acid supplementation might be effective in the regions without folate fortification [28], where the maximum benefit from folate supply to the risk of stroke is not yet achieved by folic acid supplementation interventions.

Potential adverse effects of folic acid

Cancer

Concerns have been raised that folic acid fortification might be related to the increase of cancer risk in the population. This was first highlighted in 2007 by an ecological study hypothesizing that a temporal increase in the incidence of colorectal cancer (CRC) from 1996 to 1999 was related to the introduction of folic acid fortification in the USA and Canada in 1998 [29]. This study used data from the National Cancer Institute's Surveillance and Epidemiology and End Results database to examine changes in CRC incidence rates. Authors concluded that the start of the increase in the incidence of CRC coincided with the introduction of folic acid fortification [29]. However, these conclusions are invalidated in light of the most recent evidence (see discussion below) [30, 31], suggesting that such an early increase in CRC incidence rates in the country would mean a nearly instantaneous biologic response to the folic acid fortification.

Another study that raised concerns among SACN members was an RCT that assessed the effect of folic acid supplements on the prevention of the recurrence of colorectal adenomas [32]. Authors found that folic acid supplements did not prevent recurrence of colorectal adenomas. Moreover, this study reported that the folic acid group had an increased recurrence risk of having three or more adenomas (but not one or two) [32]. Nevertheless, in 2008 two other published RCTs of folic acid supplementation did not confirm these findings [33, 34], and showed that folic acid supplementation did not increase the risk of colorectal adenomas. Furthermore, one of these studies found that folic acid supplementation did prevent the recurrence of colorectal adenomas [33]. Because of this inconsistency in findings from individual studies, there is a need to look at the systematic reviews investigating the effects of folic acid supplementation or fortification programs on cancer risk. This paper summarises the results from two most recent meta-analyses [30, 31].

A meta-analysis conducted by Vollset and colleagues [31] included 13 RCTs of folic acid supplementation involving in total 49,621 participants. The meta-analysis did not find a significant effect on overall first cancer incidence rates (RR: 1.06; 95% CI: 0.99 to 1.13; see Figure 2). No significant effect of folic acid allocation was found on the incidence of colorectal, lung, breast, prostate, any of the less known or unknown types of cancers during the first 5 years of treatment compared with placebo. Authors conclude that folic acid supplementation does not substantially increase or decrease incidence of site-specific cancers during the first 5 years of treatment. The authors also highlight that fortification of flour involves doses of folic acid that are, on average, an order of magnitude smaller than

those used in the folic acid supplementation interventions in the included studies (median daily dose of folic acid in the trials was 2.0 mg), which renders programs of flour fortification with folic acid safe with regards to cancer development [31].

The findings from the abovementioned meta-analysis are confirmed by another recent meta-analysis conducted by Qin and colleagues that included 15 RCTs of folic acid supplementation, of which 13 provided data on total cancer incidence, 6 reported total cancer mortality and 7 provided data on site-specific cancers [30]. The study demonstrated overall no significant effect of folic acid supplementation on total cancer incidence, colorectal cancer, other gastrointestinal cancers, prostate cancer, other genitourinary cancers, lung cancer, breast cancer, haematological malignancy and total cancer mortality. Moreover, significantly reduced risk of folic acid supplementation was found for melanoma [30].

With regards to pediatric cancer rates, a recent investigation was conducted to examine whether the risk of early pediatric cancers in the 2 youngest age groups changed in the province of Ontario following the introduction of folic acid flour fortification in Canada in 1998 [35]. Authors calculated incidence rate ratios (IRR) for pre- and post-fortification

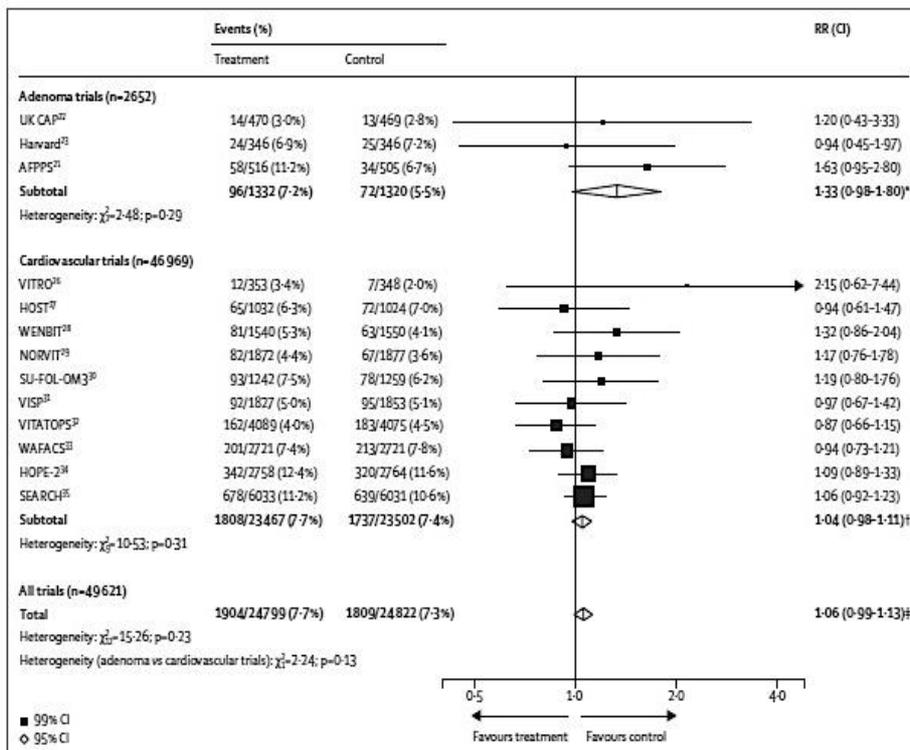


Figure 2. Effects of folic acid allocation on overall first cancer incidence [31]

Notes: RR=rate ratio. The black squares denote the RRs and horizontal lines the 99% CIs. Each square has an area inversely proportional to the variance of the log of the RR. The diamonds represent the summary estimates and their corresponding 95% CIs. * $p=0.07$. † $p=0.20$. ‡ $p=0.10$.

periods and also conducted time series analysis. The investigation did not find significant changes in the overall incidence of all cancers combined between pre- and post-fortification periods (13.47/100000 versus 13.64/100000, respectively). Significant decline in Wilm's tumor was observed after fortification for children aged 0 to 4 years (IRR: 0.74; 95% CI: 0.57 to 0.95; see Figure 3). The study did not find significant changes in any of the cancer categories for children aged 5 to 9 years [35]. The authors conclude that universal folic acid fortification did not alter the overall rates of pediatric cancer, but there may be protective effects on the risk of Wilm's tumor.

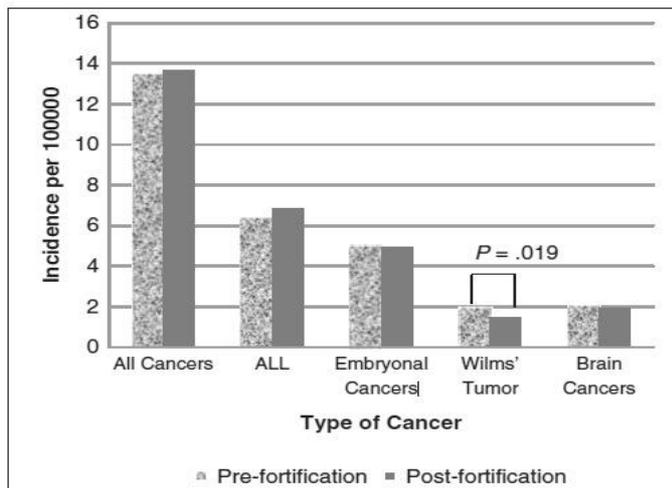


Figure 3. Incidence rates of pediatric cancers among children 0 to 4 years of age before and after folic acid flour fortification. ALL, acute lymphoblastic leukemia [35]

Vitamin B₁₂ deficiency and cognition

Because of the interrelatedness of the roles of vitamin B₁₂ and folate in the synthesis of protein, concerns are raised that folic acid might mask vitamin B₁₂ deficiency and prevent early diagnosis of anemia, therefore exacerbating neuropathy associated with vitamin B₁₂ deficiency [36]. In this regard, a study conducted by Milles and colleagues did not find any evidence of folic acid fortification masking anemia of vitamin B₁₂ deficiency [37].

A decline in cognition in the elderly is another issue that has been raised in relation to mandatory folic acid fortification and vitamin B₁₂. In 2005, a cohort study of elderly suggested that the elderly with high intake of folate demonstrated faster decline in cognitive function than those with low intake of folate [38]. Authors concluded that high intake of folate might be associated with more rapid cognitive decline in the elderly and called for further studies to validate these findings. An alternative explanation for these findings, however, is suggested in the review by Berry and colleagues, who argue that the observed faster decline could be a matter of regression to the mean, given the four-fold higher baseline levels of cognition among the elderly with high folate intake [5].

Another observational study of the elderly aged 60 years and older was conducted in 2007 using the data from 1999-2002 US National Health and Nutrition Examination Survey. The results showed association between high serum folate and anemia and cognitive impairment in the elderly with low vitamin B₁₂ status. However, for those with adequate vitamin B₁₂ status, high serum folate was associated with protection against cognitive impairment [39]. Even though this study controlled for a number of potential confounders, such as demographic characteristics, cancer, smoking, alcohol intake, serum ferritin and serum creatinine, an alternative explanation cannot be ruled out suggesting that the elderly who have problems with cognition because of vitamin B₁₂ deficiency are more likely to consume multivitamins containing folic acid, which would increase their serum folate levels and therefore create an apparent association between high serum folate levels and cognitive impairment [5]. Because of these limitations, authors suggest to rely on evidence from experimental studies. The latter, however, do not confirm the findings from these observational studies.

A two-year randomized, double blind, placebo controlled trial of homocysteine lowering interventions with B vitamins (including folate) in the elderly aged 65 and older (n = 276) did not find significant differences between the intervention and placebo groups and therefore rejects the hypothesis that folic acid supplementation inversely affects cognitive

function in the elderly [40]. Furthermore, another randomized, double blind, placebo controlled trial of folic acid supplementation in the elderly aged 50 to 70 years (n = 818) found improvements in the domains of cognitive function (i.e. memory, sensorimotor speed, complex speed, information processing speed, and word fluency) over the course of 3 years [41]. Finally, the Cochrane systematic review conducted in 2008 including 8 randomized trials did not detect any adverse effects or cognitive declines as a result of folic acid supplementation (with or without vitamin B₁₂) among unselected healthy or cognitively impaired elderly [42]. Although the authors were not able to pool the data in a meta-analysis, given the observed large heterogeneity among the studies, several of the included trials report beneficial effects of folic acid supplementation on the cognitive function of the older people [42].

A hypothetical association has been suggested between high red blood cell folate concentrations (presumably as a result of prenatal supplements containing 500 µg/100g) and development of insulin resistance in offspring later in childhood in a sample of Indian women with vitamin B₁₂ deficiency [43]. However, in a similar vein, another observational study conducted in Nepal failed to find evidence that prenatal use of micronutrients by pregnant women was associated with increased insulin resistance in later childhood [44]. Given the lack of experimental data, it is hard to draw definite conclusions.

Twinning

Another concern with regards to maternal use of folic acid supplements is the increased risk of twinning as evidenced in Sweden and Hungary [45-48]. These findings, however, are suspected to be confounded by frequent use of assisted technologies in these countries [49, 50]. In addition, this evidence is not supported by studies investigating trends in the occurrence of twin births in the US before and after the introduction of mandatory folic acid fortification; even if observed, the increase in the twinning rates is found to have started before the introduction of mandatory fortification in the country [51-55]. The recent systematic review assessing the impact of micronutrient fortification of food on women and child health (see discussion above [6]) did not find a significant effect for the pooled estimate of twinning (RR: 1.06; 95% CI: 0.92 to 1.22). Recently, it has been suggested that folate fortification might increase the success rate of in vitro fertilization (IVF), however also increasing the risk of twin births as a result of multiple embryo transfer [56].

Unmetabolized folic acid in blood

Several studies have shown that folic acid supplementation and fortification programs are associated with an increase of unmetabolized folic acid concentrations in blood [57, 58]. Even though the precise physiologic mechanisms and potential health effects of unmetabolized folic acid are yet unknown, one study has found an association between higher serum concentrations of unmetabolized folic acid and decreased natural killer cell activity in postmenopausal women [59]. This finding is however hypothetical and needs further confirmation, given other studies suggesting associations between folate deficiency and a decrease in natural killer cell activity [60, 61].

An elucidating study has been conducted recently by a team at the Institute of Food Research and Newcastle University demonstrating that contrary to the current thinking, the physiologic doses of folic acid (pterylmonoglutamic acid that is used as a dietary vitamin folate) are *not* biotransformed and processed by body in the same way as the natural circulating plasma folate (i.e. 5-methyltetrahydrofolic acid), the form found in green vegetables [62]. The investigators conducted a crossover study and were able to sample blood from subjects who were administered either a labelled folic acid or a labelled natural

folate. The results showed that 86% of folic acid in the hepatic portal vein remained unmetabolized, while almost all of the natural folate was biotransformed correctly. Consequently, the investigators call for mandatory fortification programs to use natural forms of folate, which are already licensed for use (Metafolin and Quatrefolic). If these are adopted for use in fortification programs than the potential risks from unmetabolized folic acid in the body will be reduced [62].

Conclusions and Recommendations on the level of folic acid added to flour

As reported by a folic acid working group from the Centers of Disease Control and Prevention (CDC), during the past several years a number of literature reviews of potential beneficial and adverse effects of folic acid have been conducted by food safety agencies in the countries that are considering mandatory folic acid fortification programs [5]. These agencies include: SACN for the UK Food Standards Agency [14], the Food Standards Australia New Zealand (FSANZ) [63], the Food Safety Authority of Ireland [64], and the Health Council of the Netherlands [65]. All of these agencies have approved of the folic acid fortification programs in the final reports written for their countries. Among several other potential benefits of folic acid fortification, the primary reason for its implementation is the prevention of NTDs. Meanwhile, it is worth noting that the vast majority of the concerns discussed above regarding the potential adverse effects of folic acid are associated with higher serum folate concentrations or higher intakes of folic acid that have been investigated in the context of higher dosage supplement use, rather than consumption of fortified flour products alone. The revised WHO recommendations for wheat flour fortification with folic acid according to the level of daily flour consumption are presented in Table 2.

Table 2. Average levels of nutrients to consider adding to fortified wheat flour based on extraction, fortification compound, and estimated per capita flour availability [15]

Nutrient	Flour Extraction Rate	Compound	Level of nutrient to be added in parts per million (ppm) by estimated average per capita wheat flour availability (g/day) ¹			
			<75 ² g/day	75-149 g/day	150-300 g/day	>300 g/day
Iron	Low	NaFeEDTA	40	40	20	15
		Ferrous Sulfate	60	60	30	20
		Ferrous Fumarate	60	60	30	20
		Electrolytic Iron	NR ³	NR ³	60	40
	High	NaFeDTA	40	40	20	15
Folic Acid	Low or High	Folic Acid	5.0	2.6	1.3	1.0
Vitamin B ₁₂	Low or High	Cyanocobalamin	0.04	0.02	0.01	0.008
Vitamin A	Low or High	Vitamin A Palmitate	5.9	3	1.5	1
Zinc ⁴	Low	Zinc Oxide	95	55	40	30
	High	Zinc Oxide	100	100	80	70

Notes: 1. These estimated levels consider only wheat flour as main fortification vehicle in a public health program. If other mass-fortification programs with other food vehicles are implemented effectively, these suggested fortification levels might need to be adjusted downwards as needed.

2. Estimated per capita consumption of <75 g/day does not allow for addition of sufficient level of fortificant to cover micronutrients needs for women of childbearing age. Fortification of additional food vehicles and other interventions should be considered.

3. NR = Not Recommended because very high levels of electrolytic iron needed could negatively affect sensory properties of fortified flour.

4. These amounts of zinc fortification assume 5 mg zinc intake and no additional phytate intake from other dietary sources.

FORTIFICATION OF WHEAT FLOUR WITH IRON

Iron deficiency and health consequences

Iron in the body is present in the erythrocytes as hemoglobin, where its key role is to transfer oxygen from the lungs to the tissues [1]. Iron is also important in the enzyme systems responsible for the oxidative metabolism [66]. It is stored in the liver as ferritin and as hemosiderin [1]. Iron levels in the organism are commonly assessed by hemoglobin concentrations in blood or those of ferritin in serum or plasma.

“Iron deficiency” is a condition characterized by long-term iron imbalance, and in more severe cases can cause anemia. Anemia which is caused by iron deficiency is commonly referred to as *iron-deficiency anemia*. Anemia is defined as low blood hemoglobin concentration [1]. The cutoffs of the latter that indicate anemia vary for different population subgroups (e.g. age, sex), and are defined by WHO [67]. If the prevalence of low hemoglobin concentrations exceeds 5% in the population, anemia is regarded as a public health problem. The main risk factors for iron deficiency are summarized in Table 3.

Table 3. Iron deficiency: prevalence, risks factors and health consequences (data taken from WHO guidelines [1])

Nutrient	Prevalence of deficiency	Risk factors	Health consequences
Iron	<ul style="list-style-type: none">- Estimated 2 billion cases of anemia worldwide- In developing countries the estimates are: 50% in pregnant women and infants under 2 years; 40% in school-aged children, 25-55% in other women and children- Iron deficiency is estimated to account for 50% of all anemia cases- There are about 1 billion cases of iron deficiency anemia and further 1 billion cases of iron deficiency without anemia worldwide	<ul style="list-style-type: none">- Low intakes of meat, fish and poultry and high intake of cereals and legumes- Preterm delivery of low birth weight- Pregnancy and adolescence- Heavy menstrual losses- Parasite infections causing heavy blood losses- Malaria- Low intakes of vitamin C- Allergy to cow's milk	<ul style="list-style-type: none">- Reduced cognitive performance- Lower work performance and endurance- Impaired iodine and vitamin A metabolism- Anemia- Increased risk of maternal and child mortality (with more severe anemia)

The major health consequences of iron deficiency are anemia, reduced cognitive and physical performance and maternal and child mortality [68-70]. Therefore there is substantial evidence that iron supplementation might reverse these adverse health effects. By way of illustration, a recent systematic review found that prenatal iron supplementation substantially improved birth weight [71]. Furthermore, evidence also suggests that iron supplementation benefits vitamin A and iodine metabolism [72, 73].

Evidence of the effectiveness of iron supplementation and fortification programs

With regards to the systematic reviews and meta-analyses investigating the effectiveness of flour fortification programs with iron, a protocol has been published recently for a Cochrane systematic review that will aim to examine the benefits and harms of wheat flour fortification with iron alone and with other vitamins and minerals on anemia, iron status and health-related outcomes in populations [74].

A systematic review that was conducted in 2012 aimed to evaluate the effect of iron fortified foods and biofortified crops on hematologic and biological outcomes [75]. This systematic review included 60 randomized trials, with the majority using cereal-based fortification (n=42%) and ferrous sulphate (28%) and NaFeEDTA (20%) as the most common iron fortificants. The results demonstrate that iron fortification of foods significantly increased hemoglobin (WMD: 0.42 g/dL; 95% CI: 0.28 to 0.56, see Figure 4), serum ferritin (WMD: 1.36 Ig/dL; 95% CI: 1.23 to 1.52), reduced risk of anemia (RR: 0.59; 95% CI: 0.48 to 0.71) and iron deficiency (RR: 0.48; 95% CI: 0.38 to 0.62). However, the review did not find significant effects on serum zinc concentrations, infections, physical growth and mental and motor development. Furthermore, authors conducted exploratory subgroup analyses, which revealed that the hematologic response varied with the choice of fortification vehicle and iron fortificant used: significantly higher hemoglobin response was observed with the use of salt and sauce and NaFeEDTA as an iron fortificant.

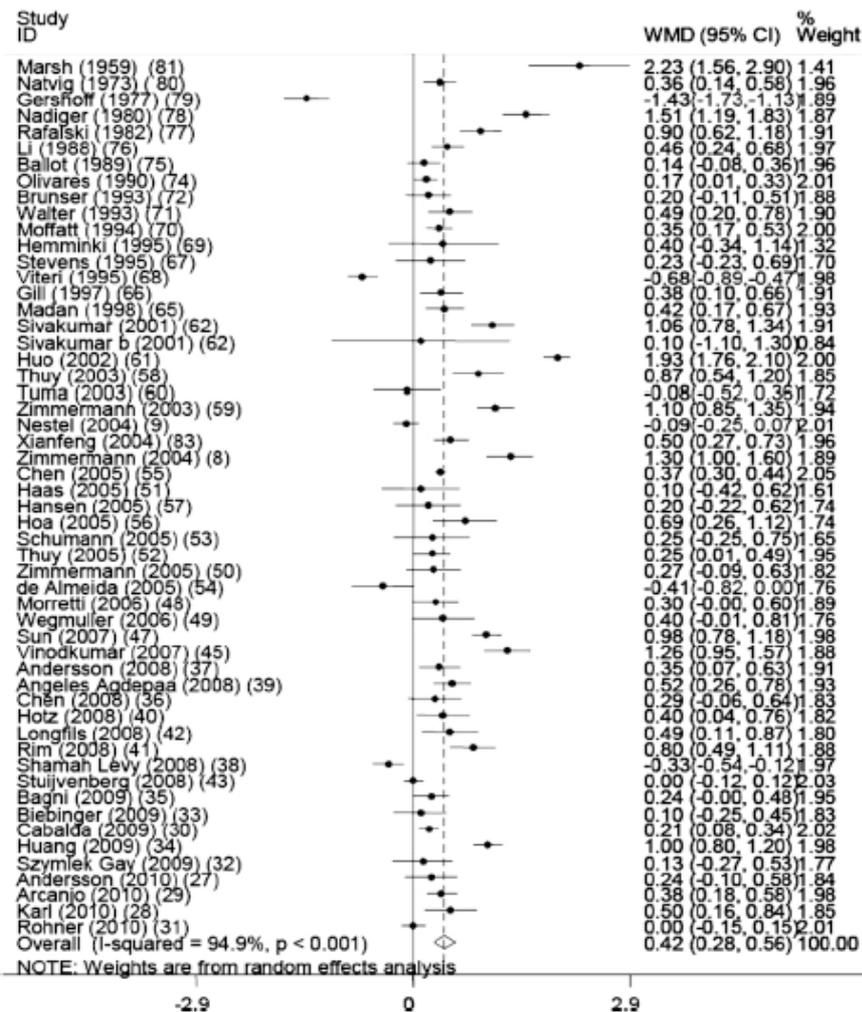


Figure 4. Forest plot for hemoglobin (after intervention) of iron-fortified foods compared with placebo.

Notes: ID – identification; WMD – weighted mean difference.

Interestingly, the duration of fortification programs was not significantly linked to the hematologic response in this review. The review suggests that a six-month fortification program might be sufficient for the hematologic response to emerge.

These effects on hematologic outcomes are confirmed by the systematic review examining the effectiveness of micronutrient fortification of food on women and child health as described above [6]. Specifically, pooled analysis from 37 RCTs showed a significant increase in hemoglobin concentration (SMD: 0.55; 95% CI: 0.34 to 0.76), serum ferritin levels (SMD: 0.92; 95% CI: 0.38 to 1.44) and reduction in anemia (RR: 0.55; 95% CI: 0.42 to 0.72) among children. The conducted subgroup analyses revealed that the impact of food fortification was stronger in populations with nutritional deficiencies at baseline. Furthermore, the effect of NaFeEDTA when used as the iron fortificant was significant on hemoglobin levels and anemia but was not significant on serum ferritin levels, while the effect of ferrous sulfate when used as the iron fortificant was significant for all the three outcomes [6]. Significant effects were also found for women for all the three outcomes assessed in a pooled analysis from 11 RCTs.

The findings from these systematic reviews are broadly in line with the evidence from iron supplementation evaluations. A systematic review that aimed to examine the evidence for health benefits and risks of preventive oral iron supplementation in children less than 5 years of age in developing countries, found increased hemoglobin concentrations in iron-supplemented children who were anemic or had iron-deficient anemia at baseline [76]. In terms of the outcomes of growth, this study showed mixed results: two studies found a positive effect of iron supplementation on height increases in iron-deficient children, while 2 studies found a negative effect and 2 found no effect on height or length in iron-replete children. Significant negative effects were also found on weight in iron-replete subgroups of young children. Because of this, authors suggest for preventive supplementation programs to specifically target iron-deficient children [76]. In any case, it is worth noting that this review focused on iron supplementation programs only and did not include studies on the impact of iron fortification.

In order to draw a comprehensive picture on current iron fortification practices worldwide, as well as to evaluate and predict their effectiveness, investigators reviewed evidence from currently available efficacy studies [77]. Authors drew required flour fortification levels for each iron fortificant based on the minimum iron dose that improved iron status in these efficacy studies (see Table 2). When compared to current wheat flour fortification programs worldwide, the authors found that most of these programs will not significantly affect iron status at the national level, because of their failure to specify a recommended iron fortificant, use of iron fortification levels that are too low in relation to the wheat flour consumption patterns of the population and little coverage [77]. Moreover, because of the low cost and good sensory properties, most of the programs use atomized or hydrogen-reduced iron powders, which however have reduced bioavailability and lack evidence for effectiveness. Therefore, the authors conclude that most of the current iron fortification programs are likely to be ineffective. Only nine national programs are found likely to significantly improve iron status if coverage is optimized. These are: Argentina, Chile, Egypt, Iran, Jordan, Lebanon, Syria, Turkmenistan and Uruguay [77]. The authors recommend legislation updates in many countries to adopt adequate levels of flour fortification and recommended iron fortificants.

The findings from the foregoing analysis elucidate the lack of iron fortification impact on anemia in urban Brazilian children [78]. Authors report that half of the surveyed mills in the country used low bioavailability (5%) reduced compounds to fortify flour. Therefore, the authors conclude: *“the national program was ineffective as implemented. This does not*

mean that optimally implemented flour fortification programs, using appropriate foods and bioavailable Fe compounds, could not be efficacious” [77, p. 1800].

Potential adverse effects of iron

The largest concern with regards to iron supplementation and fortification programs is the risk for secondary *iron overload* among certain subgroups of the fortified population. In general, there are very few reports on the risk of iron overload from supplements among individuals with normal phenotypes [77]. Iron overload mainly occurs among individuals with iron metabolism disorders, which disturb normal iron absorption and/or storage. The most common iron overload disorder is associated with mutations in the HFE gene, which is responsible for hereditary hemochromatosis. Physiological conditions, such as thalassemia, pyruvate kinase deficiency, and glucose-6-phosphate dehydrogenase deficiency are also associated with iron overload [79]. Among the acquired conditions that are associated with iron overload are sideroblastic anemia, or any anemia except for that due to blood loss, in which multiple transfusions are required [80]. Although men do not have a mechanism of iron loss, which puts them at higher risk of accumulating iron as a result of consuming iron fortified foods over a long period of time, a study that examined long-term consequences of iron-fortified flour consumption in Iran did not find increased risk of iron overload in men [81]. Another investigation, however, suggests a potential decrease in antioxidant capacity in men [82]. This evidence needs further testing.

As in case of iron overload, there have been concerns raised on theoretical grounds over the harmful effects of iron therapy and supplementation with regards to infections. A systematic review that included 28 RCTs involving 7892 children of oral or parental iron supplementation or fortified milk and cereals did not detect a significant effect on the overall incidence of infectious illnesses in children with a slight increase in the risk of diarrhea [83]. Furthermore, a recent review on oral supplements for children in malaria-endemic areas concluded that iron alone or with antimalaria treatment does not increase the risk of clinical malaria or death when regular malaria surveillance and treatment services are in place [84]. It is worth noting that these evaluations focused only on iron supplementation programs; infections along with other adverse health outcomes are planned to be assessed in two systematic reviews of wheat and maize flour fortification programs, the protocols of which are already published [74, 85].

Conclusions and recommendations on the level of iron added to flour

The first global recommendations for the type and level of different iron fortificants were made by a CDC expert group in Cuernavaca in 2004. They proposed a pragmatic procedure that followed the method recommended by WHO [1]. According to this method each country needs to first measure the daily iron intake in the groups at risk for iron deficiency, estimate the iron bioavailability from the diet, compare these two and calculate the corresponding iron amount lacking in the diet. This amount should then be added to the mean daily flour consumption of the targeted groups.

Because of the lack of capability of many countries to follow the abovementioned procedure, these recommendations were subsequently revised based on the evaluation of the published efficacy studies of wheat flour fortification programs (see the discussion above) [77]. It is proposed that iron fortification of wheat flour is considered at the national or regional levels only if laboratory evidence suggests a high prevalence of iron deficiency and iron-deficiency anemia in women and children in the country (iron-deficiency anemia rate of >5%). Iron fortification programs should aim to decrease the prevalence of iron deficiency in the target at-risk populations and achieve <10% prevalence of iron deficiency

and <5% of iron-deficiency anemia [86]. These should be reached in 2 to 3 years after the start of the fortification program.

According to these revised recommendations, countries are expected to first test the recommended levels of iron fortificants in both flour and final products made from fortified flour to check for the acceptability of any potential sensory changes. The first choices as iron fortificants for wheat flour fortification are NaFeEDTA, ferrous sulfate and ferrous fumarate. Electrolytic acid is considered the second-choice iron compound for wheat flour fortification, and because of the uncertainty regarding the lowest effective dose of electrolytic iron the recommendations on its levels are not changed from the Cuernavaca Workshop (see Table 2) [77].

The greatest confidence in the revised recommendations for iron fortification with wheat flour is placed on NaFeEDTA because its absorption is less likely to be affected by other components of the meals in which it is eaten [77]. It is the only iron compound recommended for the fortification of high-extraction wheat flour (>0.8% ash). Additionally, the higher iron bioavailability from wheat-based foods fortified with NaFeEDTA gives an opportunity for lower levels of iron fortification, which in turn reduces the risk for sensory changes. Finally, there is evidence suggesting that NaFeEDTA may promote liquid oxidation in stored wheat flour [87].

There is no evidence supporting the use of hydrogen-reduced iron powders or atomized reduced iron powders for wheat and maize flour fortification. These compounds are less well absorbed than electrolytic iron and therefore are not recommended for wheat flour fortification [77].

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