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Wheat flour fortification and human health

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1 Introduction

The objective of this chapter is to review evidence of the human health impact of wheat flour fortification.

1.1 What is wheat flour fortification?

Food fortification is the addition of nutrients to foods while they are being processed (WHO and FAO 2006). Also known as enrichment, food fortification is a unique intervention in health circles because it is delivered by the private sector in contrast to most public-health interventions that are implemented by the public sector. For wheat flour, fortification occurs in a mill after parts of the wheat kernel are ground to flour: the endosperm in refined white flour and the bran, germ and endosperm in whole-grain flour (Bauernfeind and DeRitter 1991). Small concentrations of nutrients, usually vitamins and minerals, are added to this flour in the mills. Wheat flour can be fortified in large, industrial-sized mills or in small, non-industrialized mills. This chapter will focus on

http://dx.doi.org/10.19103/AS.2021.0087.23

large-scale industrial fortification of wheat flour where most of the evidence and success with flour fortification is observed.

With a few exceptions, most nutrients can be added to wheat flour through fortification (WHO and FAO 2006). Nutrients in the outer layers of the wheat kernel that were removed during milling can be added to wheat flour through fortification. The B vitamins thiamin, riboflavin and niacin are examples of this (Bauernfeind and DeRitter 1991); adding these nutrients back to flour through fortification is known as restitution or restoration. Nutrients can be added back at the same, lower or higher levels than present in the kernel. Nutrients that are not naturally found in the wheat kernel can also be added to flour through fortification. Vitamin B12 is an example of this (USDA 2020).

Nutrients are usually added through fortification coupled with another ingredient(s). For example, iron can be added in the form of ferrous sulfate or sodium iron ethylenediaminetetraacetate (NaFeEDTA) (WHO 2009); these forms are known as fortificants or fortification compounds. Niacin can be added in the form of niacinamide, nicotinic acid or nicotinamide (WHO and FAO 2006). Because nutrients are added to flour to benefit human health, forms that are better absorbed by the human body (i.e. more bioavailable) are preferred. They may be costlier than less well-absorbed forms (e.g. NaFeEDTA compared with electrolytic iron); however, less needs to be added of the more bioavailable form to have a comparable health benefit (Hurrell et al. 2010).

There are different reasons why some nutrients or fortificants are not typically added to wheat flour through fortification. For bioavailability purposes, another food may be a better choice to add the nutrient to; this is the case with vitamin A. Vitamin A requires fat for absorption; oil, margarine and butter are better options for fortifying because they are lipid rich. Vitamin A can be added to flour; however, it is a costly ingredient because of the processing required to encapsulate the vitamin so it can be mixed into flour (WHO and FAO 2006). A fortificant may interfere with the technological processing or sensory characteristics of the food made with fortified flour. For example, ferrous sulfate can cause rancidity in high-fat foods (WHO and FAO 2006). For these foods, a less reactive iron compound may be used in flour fortification.

1.2 Why fortify wheat flour?

Many countries with mandatory fortification document their reason for fortifying wheat flour (Marks et al. 2018). It is to address a widespread health problem caused by a nutrient deficiency(ies) in the population, such as iron deficiency, anemia and neural tube defects.

The reason wheat flour is chosen to fortify is because it meets two basic criteria. First, food made with wheat flour (such as bread, noodles, pasta) is consumed by a large proportion of the population trying to be reached.

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Second, most of the world's wheat flour is produced in large-scale, industrial mills (FFI 2020). This is important because large-scale fortification is easier for industry to implement and for the government to monitor compared with small-scale fortification (WHO and FAO 2006).

2 Status of wheat flour fortification

The status of wheat flour fortification can be organized by three non-mutually exclusive categories: countries with foundational documents that establish a wheat flour fortification program; countries with documented performance of existing flour fortification programs; and countries without fortification programs which have the potential to benefit from wheat flour fortification. Country statistics on these three categories of flour fortification can be found at the Global Fortification Data Exchange website (FortificationData.org). This chapter will focus on a subset of countries with 'foundational documents' – those with legislation that mandates or allows voluntary fortification and those with standards for wheat flour fortification – and documented performance of their flour fortification programs.

2.1 Countries that mandate wheat flour fortification

As of February 10, 2020, 83 countries have legislation that effectively mandates the fortification of wheat flour (Global Fortification Data Exchange 2020a). This means the country has 'documentation [which] indicates that fortification of all or some of the food is compulsory or required'. These countries are shown in green in Fig. 1.

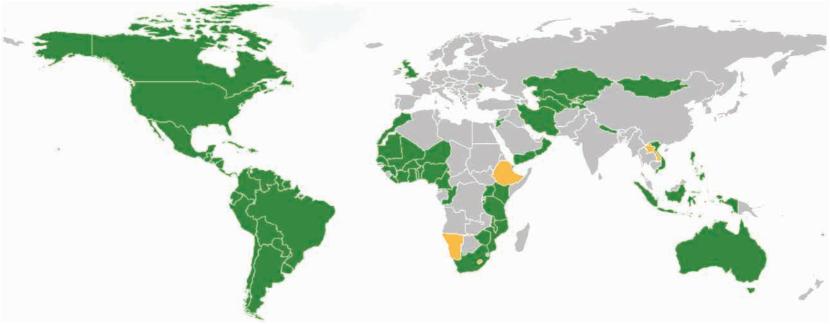

Figure 1 Countries in green are those with legislation that has the effect of mandating wheat flour fortification with one or more nutrients (Global Fortification Data Exchange 2020a). Countries in yellow are those confirmed to not have mandatory legislation of wheat flour. Countries in grey are those unlikely to have mandatory fortification of wheat flour; however, this information has not been confirmed by an in-country contact.

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Countries with mandatory wheat flour fortification share the following characteristics:

- Following the United Nations' 2018 regional designation (United Nations Statistics Division 2020), 25 are in the Africa Region, 35 are in the Americas region, 16 in the Asia region, three are in the Europe region and four in Oceania.
- Sixteen are low-income countries, 29 are lower-middle-income countries, 25 are upper-middle-income countries and 13 are high-income countries per the World Bank's 2017 designation (World Bank 2020).
- Sixteen countries also have mandatory fortification of maize flour (Global Fortification Data Exchange 2020a).

2.2 Countries that allow voluntary fortification of wheat flour

As of February 10, 2020, 14 countries have 'official documentation and/or a food standard that provides guidance or regulations for fortification but does not have the effect of mandating or requiring fortification', that is, voluntary fortification of wheat flour (Global Fortification Data Exchange 2020a). These countries are shown in blue in Fig. 2.

2.3 Countries with standards for wheat flour fortification

Standards are documents that 'indicate standardized fortification levels of the food vehicle in question with one or more nutrients' (Global Fortification Data Exchange 2020b). Among the 97 countries with mandatory or voluntary wheat flour fortification, the Global Fortification Data Exchange has standards for 91. Table 1 lists the nutrients that are included in these 91 standards (Global

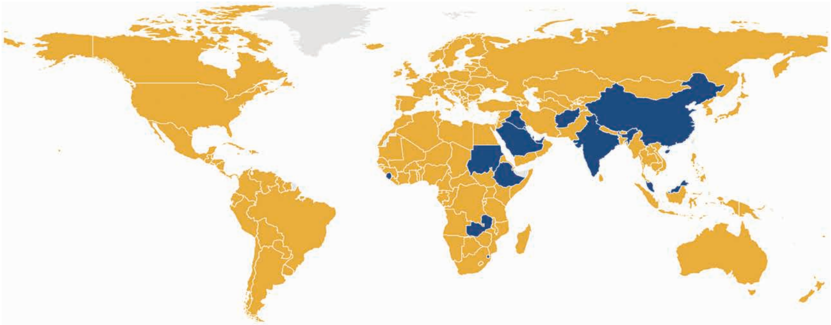

Figure 2 Countries in blue have voluntary wheat flour fortification with one or more nutrients (Global Fortification Data Exchange 2020a). Countries in yellow do not have voluntary fortification of wheat flour.

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Table 1 Among 91 countries with mandatory or voluntary wheat flour fortification whose standards are available, the nutrients and amounts that are listed in fortification standards (Global Fortification Data Exchange 2020b)

Nutrient	Number of countries	Amount (mg/kg)
Iron	89	15–120
Folic acid (vitamin B9)	73	0.1–5.11
Thiamin (vitamin B1)	66	1.25–10
Riboflavin (vitamin B2)	64	1.3–6.6
Niacin (vitamin B3)	64	6.7–60
Zinc	31	12.5–101.3
Calcium	23	1.28–2,400
Vitamin A	20	0.62–10
Vitamin B12	20	0–0.04
Vitamin B6	14	2.0–6.5
Vitamin D	7	0.01–0.02
Selenium	1	0.21

Fortification Data Exchange 2020b). Overall, standards include up to four different minerals and eight different vitamins.

Most countries include iron, folic acid, thiamin, riboflavin and niacin in their standards for wheat flour fortification. Thirty one or fewer countries include zinc, calcium, vitamin A, vitamin B12, vitamin B6, vitamin D or selenium in their standards.

3 How the human health impact of wheat flour fortification is measured

The remainder of the chapter will center on health improvements observed from large-scale fortification of wheat flour, either alone or in combination with maize flour.

There are two types of studies to assess if food fortification has a health impact. The first are efficacy studies which tell us the 'extent to which [fortification] produces a beneficial result under ideal conditions' (Samet et al. 2008). Usually, efficacy is based on the results of randomized controlled trials. In comparison, effectiveness studies estimate the extent to which fortification produces a beneficial result 'when deployed in the field in the usual circumstances'. Evidence is needed from efficacy studies to ensure that fortification can have a beneficial impact. Additionally, effectiveness studies report if these benefits are observed when programs are implemented under real-life conditions.

This chapter will focus on effectiveness trials. This is because there is good evidence from efficacy trials that if people deficient in a nutrient consume a food fortified with that nutrient, their nutrient status will improve as will their health (e.g. Muthayya et al. 2012; Black et al. 2012). What is less known is how fortification operates under real-life conditions such as when it is offered through a government's social program or it is provided through the open market.

4 Examples of health outcomes associated with wheat flour fortification that have been studied

Generally speaking, when people consume a nutrient provided from any source (such as a non-fortified food, a fortified food, a supplement), they will experience an increase in their bodies' levels of that nutrient. This improvement in nutritional status can in turn improve functional outcomes: 'nutrient-dependent physiological functions' that can be measured (Solomons and Allen 1983). For example, we expect that when people consume folic acid from any source, it will increase their blood folate levels (Fig. 3). In women of childbearing age, this will lead to a reduction in neural tube defects, a functional outcome.

Specific to fortified wheat flour, researchers have assessed its role in affecting biological markers and functional outcomes; all of these results will be reviewed in this chapter.

4.1 Biological markers of nutritional status

Biological markers of nutritional status can be measured in a minimally invasive way from biological fluids and tissues such as blood, urine and hair (Gibson 1990). In assessing the impact of wheat flour fortification in effectiveness studies, only blood and breast milk samples have been taken (Table 2).

Folate provided through the fortification compound, folic acid, is the most studied nutrient added to fortified flour. Several biological markers have been assessed such as the concentration of plasma or serum folate and red blood cell folate; these were used to quantify the prevalence (or percentage) of folate deficiency and when combined with hemoglobin, to determine the prevalence

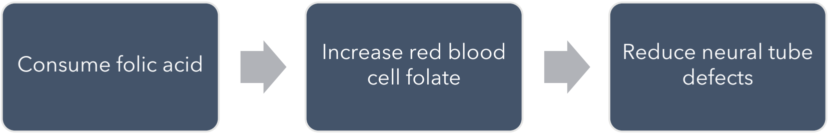

Figure 3 A representation of how fortification with a nutrient such as folic acid can lead to improvement in a biological marker of that nutrient such as red blood cell folate. In turn, an increase in red blood cell folate can lead to improvement in a functional outcome: for example, a reduced risk of neural tube defects.

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Table 2 Biological markers of nutritional status and functional outcomes assessed in studies of wheat flour fortification's impact on human health

Nutrient provided through fortification	Biological markers of nutritional status[a]	Functional outcomes[a]
Folate	Folate deficiency Folate-deficiency anemia High homocysteine Plasma/serum folate Plasma/serum homocysteine Red blood cell folate	Anemia Cancer Cognitive function Congenital heart disease Coronary heart disease Hypersensitivity Masking of vitamin B12 deficiency Myocardial infarction Orofacial clefts Neural tube defects Stroke Thyroid- and diabetes-related disorders
Iron	Body iron stores Plasma/serum ferritin Iron deficiency Iron-deficiency anemia Soluble transferrin receptor	Anemia
Zinc	Plasma/serum zinc Zinc deficiency	None assessed
Vitamin B12	Breastmilk vitamin B12 Plasma/serum vitamin B12 Vitamin B12 deficiency	None assessed
Multiple	Hemoglobin	Anemia[b]

[a] Some of the markers of nutritional status measure the concentration of nutrients or other constituents in the blood or breastmilk. Some of the markers of nutritional status and most of the functional outcomes refer to the prevalence (or percentage) of people who have the condition. The functional outcomes refer to the incidence (or number of people newly diagnosed with the condition), prevalence (or percentage of people who have the condition) or deaths (or number of people who die due to the condition).

[b] Some studies assessed the effect of fortification with multiple nutrients on the prevalence of anemia, without evaluating the contribution of fortification in improving nutrient-specific biological markers of nutritional status (for example, Assunção et al. 2007).

of folate-deficiency anemia. For iron, zinc and vitamin B12 added to wheat flour, studies assessed their impact on biological markers, as well (Table 2).

4.2 Functional outcomes

The impact of wheat flour fortification on several functional outcomes was studied (Table 2). For example, positive functional outcomes evaluated were reductions in neural tube defects and anemia while negative functional outcomes assessed were masking of vitamin B12 deficiency and an increase in cancer incidence (Table 2). Some of these outcomes are described further.

4.2.1 Neural tube defects

Neural tube defects are a type of congenital anomaly that affects the development of a baby's spine and brain while *in utero* (Avagliano et al. 2018). It is estimated that between 213 800 and 322 000 babies are born with neural tube defects around the world every year (Blencowe et al. 2018). For healthy spine and brain development, the neural tube must close by 28 days after conception (van Gool et al. 2018); this developmental milestone in the fetus occurs before most women know they are pregnant (Martinez et al. 2018).

The two most common forms of neural tube defects are spina bifida and anencephaly (Avagliano et al. 2018). Spina bifida is when the baby's spine is not formed correctly. Spina bifida can be treated, but it cannot be cured, and individuals with spina bifida have varying degrees of permanent disability for the rest of their lives. Anencephaly is when the brain is not formed correctly. All babies with anencephaly die *in utero* or shortly after birth.

With adequate folate status in women before conception, a healthy neural tube forms in the fetus (Martinez et al. 2018). Folic acid is a form of vitamin B9 that is well absorbed by the body. It can be provided in pill form or as a fortification compound (IOM 1998). Folic acid consumed by women before conception and in the first few weeks after conception prevents around 70% of these birth defects (Czeizel and Dudás 1992; MRC Vitamin Study Research Group 1991). For this reason, women capable of becoming pregnant are recommended to increase their folic acid intake by consuming supplements with folic acid, foods fortified with folic acid and foods rich in food folates (a form of vitamin B9 that the body does not absorb as well as it absorbs folic acid) (Institute of Medicine 1998).

4.2.2 Anemia

Anemia is 'a condition in which the number of red blood cells or the hemoglobin concentration within them is lower than normal' (WHO 2020). An estimated 800 million women and preschool children worldwide have anemia (Stevens et al. 2013). In public-health practice, anemia is determined by measuring hemoglobin levels in blood (Chaparro and Suchdev 2019). If the value is below a cut-off, a person is considered to be anemic. Anemia has multiple causes, both nutritional and non-nutritional in nature. Dietary deficiencies in the nutrients iron, copper, zinc, folate, vitamin B12, riboflavin, vitamin B6, thiamin, vitamin A and vitamin E – which contribute to hemoglobin synthesis – can cause anemia (Kraemer and Zimmermann 2007). Non-nutritional causes of anemia include malaria, hemoglobin disorders such as thalassemia and chronic inflammation (Chaparro and Suchdev 2019).

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The prevalence of anemia can only be reduced if the causes of the anemia are addressed. In some world regions, there are both nutritional and non-nutritional causes of anemia (Kassebaum et al. 2014). In these cases, fortification with nutrients involved in hemoglobin synthesis can only reduce the occurrence of anemia if there is a deficiency in these nutrients in the diet.

4.2.3 Masking of vitamin B12 deficiency

A concern emerged in the mid-1900s related to both folate and vitamin B12. Folate deficiency independently causes megaloblastic anemia; that is anemia where the red blood cells are larger than normal (IOM 2000). Vitamin B12 deficiency also independently causes megaloblastic anemia. Additionally, vitamin B12 deficiency causes potentially irreversible neurological conditions such as 'memory loss, disorientation and frank dementia'.

The masking of vitamin B12 deficiency occurs in a specific situation where a person has megaloblastic anemia due to vitamin B12 deficiency only (Berry 2019). This is often observed in older adults who are unable to absorb vitamin B12 from the diet as well as they did when they were younger (Allen et al. 2018). In these individuals, if folic acid is provided, the anemia is corrected. However, if vitamin B12 is not provided, vitamin B12 deficiency can persist and with it, potentially irreversible neurological conditions.

When folic acid corrects megaloblastic anemia while not treating the underlying vitamin B12 deficiency, it is known as 'folic acid masking of vitamin B12 deficiency'. Fortification with folic acid may mask vitamin B12 deficiency.

4.2.4 Cancer

Folic acid is reported to both prevent and cause cancer (Smith et al. 2008). Specifically, folic acid 'may protect against the initiation of cancer, but facilitate the growth of preneoplastic [pre-cancerous] cells'. The cancer research conducted with folic acid has mainly focused on folic acid delivered through large-dose supplements, and not lower-dose food fortification. Evidence of wheat flour fortification with folic acid causing cancer is reviewed in this chapter.

5 Additional considerations when assessing the health impact of wheat flour fortification

There are several challenges with assessing the health impact of food fortification programs through effectiveness studies. The first five of these issues can affect the interpretation of the research results; the last issue is to address the paucity of such data from countries that implement fortification programs. Potential solutions for overcoming these challenges are noted.

5.1 Lack of a control group

Because large-scale fortification is often implemented under a national mandate, it rarely offers an opportunity to have a randomly selected control group that does not get fortification for a period of time. The lack of such a group makes it difficult to infer causality for fortification (Victora et al. 2004). Thus, we cannot state with certainty that fortification causes an improvement in a health outcome.

For example, using two national surveys from Costa Rica, researchers observed there was a lower prevalence of iron deficiency, anemia and iron-deficiency anemia in children in 2008 compared with 1996 (Martorell et al. 2015). Between the two surveys, maize flour and milk were mandated to be fortified with iron, and the iron compound used to fortify wheat flour was changed to a fortificant that the body absorbs well (i.e. ferrous fumarate). Is it plausible that fortification contributed to the health impact observed?

The investigators generated a program-impact pathway of various factors in Costa Rica's food fortification program (Fig. 4) (Martorell et al. 2015). First, they assessed whether there was a potential to benefit from food fortification. They concluded there was a potential to benefit because micronutrient deficiencies were present in 1996 (27% of children were iron deficient). Next, they assessed if a fortification policy had been created and legislation passed. The answer was yes. Then, they assessed if bioavailable fortificants were mandated. The answer was also yes. Next, they determined if foods were fortified at mandated levels.

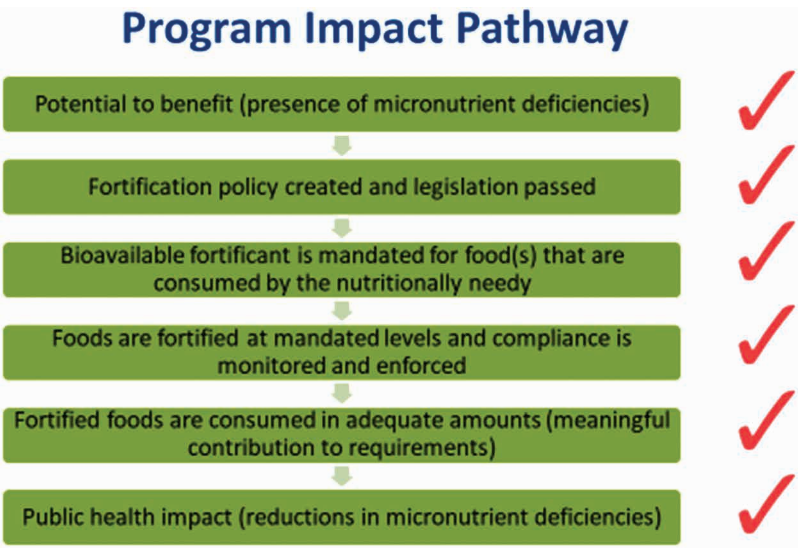

Figure 4 Program impact pathway developed by researchers to determine the plausibility of food fortification improving health outcomes (Martorell et al. 2015).

They obtained data from the government regulatory agency that confirmed that all 246 wheat flour samples obtained in bakeries over a one-year period met or exceeded the iron content required by law. After, they evaluated if fortified foods were consumed in adequate amounts. They analyzed dietary data and estimated that fortified foods contributed 49% of children's dietary iron requirement. Finally, they assessed the public health impact and saw a reduction in biological makers and a functional outcome. The affirmative responses to all of these questions suggest that it is plausible that food fortification with iron in Costa Rica contributed to the health impacts observed.

This type of complementary, program-related information can be presented for any program to argue for fortification's contribution to health impacts. For example, program decision makers can compile and triangulate information generated through government monitoring, such as compliance with fortification (Smarter Futures no date). Unfortunately, this type of information is rarely presented in effectiveness studies (Pachón et al. 2015).

5.2 Challenges of using birth defects registry data

The following experience from Peru highlights the importance of verifying electronic birth defects registry information with a review of clinical records, to minimize misclassification errors. In 2012, Ricks et al. (2012) published an article that evaluated the impact on neural tube defects (NTDs) of wheat flour fortification with folic acid which was decreed in Peru in 2005. Their work showed no reduction in NTDs in a large maternity hospital in Lima, after folic-acid fortification of wheat flour began; the pre-fortification NTD estimates (18.4/10 000 live and still births) were from 2004 to 2005 and the post-fortification NTD estimates (20.0/10 000 live and still births) were from 2007 to 2008. Electronic registry data were used to generate the NTD estimates.

Tarqui-Mamani (2013) wrote a letter to the editor of the journal that published Ricks' paper. Tarqui-Mamani's research team used the same data as Ricks; however, they reviewed clinical charts and found that 32.9% of cases in the electronic registry noted as NTDs were in fact other congenital anomalies. This suggests that the Ricks' paper overestimated the number of NTD cases in both the pre- and post-fortification periods.

In 2013, Tarqui-Mamani was part of a research team that reported their analysis of the same electronic registry data as Ricks, but after having double-checked the clinical charts (Sanabria Rojas et al. 2013). They reported on data collected in longer pre- (2001–2005) and post-fortification periods (2006–2010). The birth prevalence of NTDs that they reported in both the pre-fortification (2005: 13.6/10 000) and post-fortification (2010: 7.6/10 000) periods were lower than what Ricks reported, suggesting again, that Ricks misclassified congenital anomalies as NTDs. The Sanabria results indicate a lower prevalence of NTDs in the post-fortification period compared with the pre-fortification period.

5.3 Selecting an outcome indicator that is only responsive to the nutrients added through fortification

As noted earlier for anemia, there are many factors that together or in isolation can cause low hemoglobin levels; these include nutritional and non-nutritional causes. When anemia is the sole outcome studied to measure the impact of a nutrition intervention such as fortification, one can never be completely sure if the change (or lack of change) was due to the nutrients delivered. It is preferable to select an outcome that is directly and exclusively changed in the human body because of a particular nutrient that is provided through fortification. Examples of such outcomes are the biological markers described previously (Table 2).

5.4 Allowing sufficient time before measuring outcomes

There are two main reasons why a minimum amount of time is needed between the start of fortification program implementation and the measurement of health outcomes. One is that programs need sufficient time to ensure a consistent supply of adequately fortified food reaches the target population (Smarter Futures no date). Mills that have never fortified need time to purchase and install feeders, purchase vitamins and minerals, purchase bags with new nutrient labels, train mill staff in adding nutrients and testing for this addition, and in documenting the fortification process during all shifts. At the same time, governments need time to train inspectors in auditing mill activities and integrating inspections for fortification into existing protocols and schedules. Additionally, there may be several months between the production of flour and its appearance in the market for consumers or in mass-produced products that use flour as an ingredient. Another reason is that some biological markers and most functional indicators require a longer period of time before their presence can be measured in the human body. Neural tube defects are measured at the end of a nine-month pregnancy period and cancers can take decades to manifest (Keum and Giovannucci 2014).

For programs that are evaluated ‘too soon’ after initiation, a lack of impact can be due to either of the aforementioned reasons or to the program being ineffective (e.g. wrong food was chosen to be fortified; wrong nutrients, levels or fortification compounds selected). In Brazil, the first published studies that assessed hemoglobin levels and the prevalence of neural tube defects (NTDs) showed no difference between the pre- and post-fortification periods (Table 3), whereas later studies did observe higher hemoglobin levels and lower prevalence of NTDs in the post-fortification period.

Table 3 Studies from Brazil that reported hemoglobin levels and neural tube defects before and after fortification of wheat and maize flour

Study	Outcome	Results
Assunção et al. 2007	Hemoglobin	Hemoglobin levels are the same in pre- and post-fortification periods
Fujimori et al. 2011	Hemoglobin	Hemoglobin levels higher in the post- than pre-fortification period
Assunção et al. 2012	Hemoglobin	Hemoglobin levels higher in the post- than pre-fortification period
Pacheco et al. 2009	Neural tube defects	NTD prevalence is the same in pre- and post-fortification periods
López-Camelo et al. 2010	Neural tube defects	Prevalence of one type of NTD is the same in pre- and post-fortification periods: total spina bifida; prevalence of two types of NTDs lower in the post-fortification than pre-fortification period: anencephaly, cephalocele
Pacheco Santos et al. 2016	Neural tube defects	NTD prevalence lower in post-fortification than pre-fortification period

For these reasons, it is prudent to measure program-performance information such as the percentage of flour produced that is adequately fortified (i.e. compliance) and the percentage of people consuming adequately fortified flour (i.e. coverage) before embarking on an impact evaluation. Programs that are not delivering adequately fortified food to most of the target population are unlikely to see a health impact; in those cases, program performance should be improved before assessing impact.

5.5 Unethical to conduct randomized controlled studies for some outcomes

A randomized controlled trial would unequivocally answer the question 'does consumption of folic acid-fortified flour by pregnant women cause a reduction in neural tube defects?' However, because it has been established that folic acid delivered to pregnant women (in a supplement) reduces the first occurrence and recurrence of neural tube defects, it would be unethical to conduct such a trial where a group of pregnant women would knowingly be deprived of folic acid (Oakley 2009). For this reason, only observational studies, like those described in this chapter, can be ethically completed. Conclusions from these studies can be strengthened with program-performance data as noted earlier.

5.6 Impact evaluation surveys can be costly

Because stand-alone, fortification impact evaluation surveys can be costly, one solution is to use existing data to assess the health impact of flour fortification.

For example, in June 2004, wheat and maize flour fortification with iron and folic acid became mandatory in Brazil (Global Fortification Data Exchange, 2020c). Researchers based in the city of Recife were interested in determining if the fortification mandate had an impact on the number of babies born with neural tube defects (Pacheco et al. 2009).

Brazil has a National Information System on Live Births (Pacheco et al. 2009). The information in this system was used to determine if a child was born in Recife with a neural tube defect. The researchers then counted the number of babies born with neural tube defects before fortification became mandatory and after fortification became mandatory.

This research project did not require primary data collection by the researchers. They were able to use existing data to assess if fortification had a health impact. This and the study from Costa Rica (Martorell et al. 2015) provide a valuable lesson. Existing data, such as national nutrition surveys and live births registries, can be used to estimate the health impact of flour fortification.

Another solution for minimizing the cost of evaluating health impact is to add fortification-relevant questions to existing data-collection systems. For instance, for the 2014 Demographic and Health Survey conducted in Cambodia, decision makers added a micronutrient module for the first time (National Institute of Statistics et al. 2015). This allowed for nationally representative information to be available for several biological markers of nutrient status: iron, vitamin A, vitamin D, calcium, folate, vitamin B12 and iodine status. The resources required to add blood and urine sampling to existing surveys, such as this one, are substantially lower than paying for a stand-alone survey to exclusively measure the health impact of food fortification.

6 Health impact results observed from wheat flour fortification studies

Researchers have employed different study designs to assess if wheat flour fortification affects any of the health outcomes described in Table 2: biological markers of nutritional status and functional outcomes (both positive and negative). What follows are the trends observed from these studies (Figs. 5–7).

Nutrients and health outcomes studied:

- Folic acid is the nutrient added to fortified wheat flour that was most studied, followed distantly by iron, vitamin B12 and zinc.
- Numerous health outcomes were studied. For folic acid, these can be grouped into the following categories: folate status (including folate deficiency and folate-deficiency anemia), neural tube defects (including various sub-types), cancer (including breast, colorectal and pediatric

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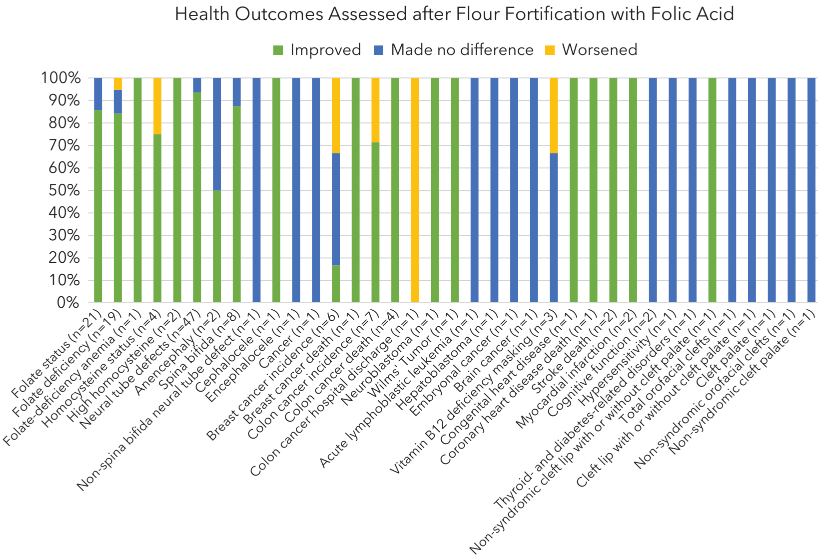

Figure 5 A summary of research on all health outcomes assessed after flour fortification with folic acid: fortification improved, worsened or made no difference in the health outcome. The number in parentheses reflects the number of analyses conducted. If studies reported overall results only, the number reflects the number of studies. If a study reported results by different population groups (e.g. women, children), the number reflects the number of population groups. 'Folate status' as reflected in serum, plasma or red blood cell levels. 'Folate deficiency' as reflected in serum, plasma or red blood cell levels below a cutoff. 'Plasma homocysteine concentration increases when inadequate quantities of folate are available to donate the methyl group that is required to convert homocysteine to methionine' (Institute of Medicine 1998). Serum or plasma homocysteine levels above a cutoff reflect folate deficiency. For 'colon cancer incidence, death and hospital discharge', this may refer to colon cancer alone or to colorectal cancer. Hypersensitivity outcomes include 'asthma, allergy and atopic disease, wheeze, hypersensitivity test, eczema and food allergy' (National Toxicology Program 2015).

cancers), homocysteine status (including high homocysteine), orofacial clefts, heart health (coronary heart disease, stroke, myocardial infarction) and others (vitamin B12 deficiency masking, congenital heart disease, cognitive function, hypersensitivity, and thyroid- and diabetes-related disorders).

- Health outcomes studied for added iron included iron status, iron deficiency and iron-deficiency anemia.
- Health outcomes studies for vitamin B12 and zinc were status and deficiency biomarkers.
- Hemoglobin and anemia, biomarkers potentially linked to multiple nutrients, were also studied.

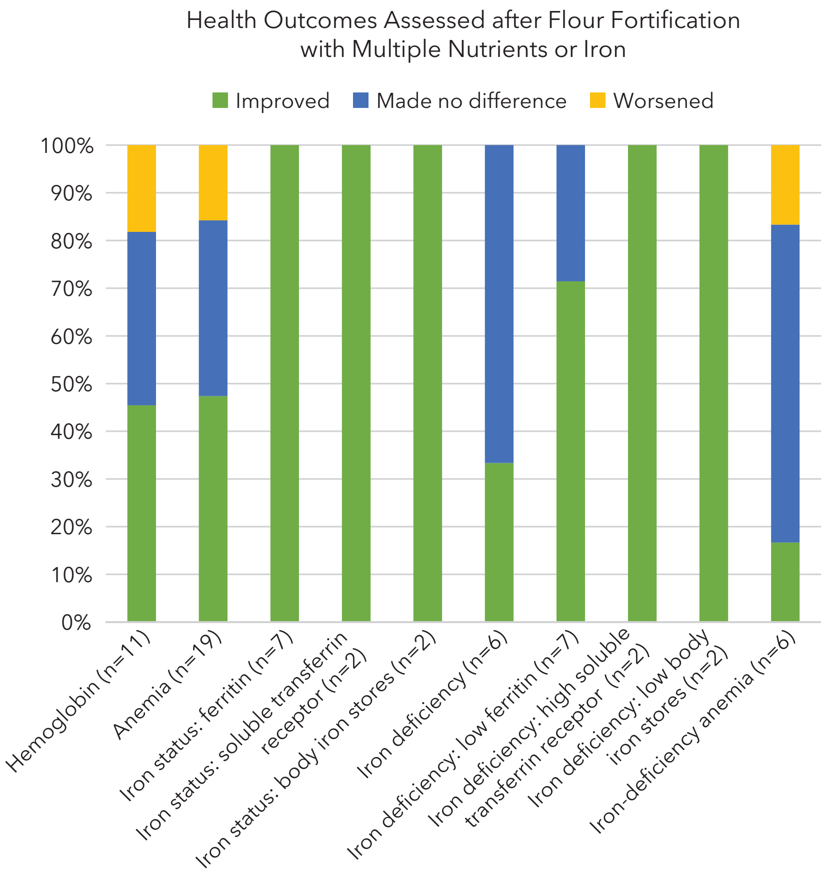

Figure 6 A summary of research on health outcomes assessed after flour fortification with multiple nutrients or iron: fortification improved, worsened or made no difference in the health outcome. The number in parentheses reflects the number of analyses conducted. If studies reported only the overall results, the number reflects the number of studies. If a study reported results by different population groups (e.g. women, children), the number reflects the number of population groups. Hemoglobin level and prevalence of anemia can be affected by multiple nutrients; they can also be affected by non-nutritional factors such as malaria infection. 'Iron deficiency' as defined by the authors.

Results from the most studied outcomes:

- The most studied outcomes were neural tube defects, cancer, folate status, folate deficiency, anemia, iron deficiency, iron status, hemoglobin and iron-deficiency anemia.
- Except for iron-deficiency anemia, most analyses showed improvements in all of these outcomes after fortification. For example, cases of neural tube defects and cancers overwhelmingly decreased and levels of folate and iron in biomarkers increased after fortification.

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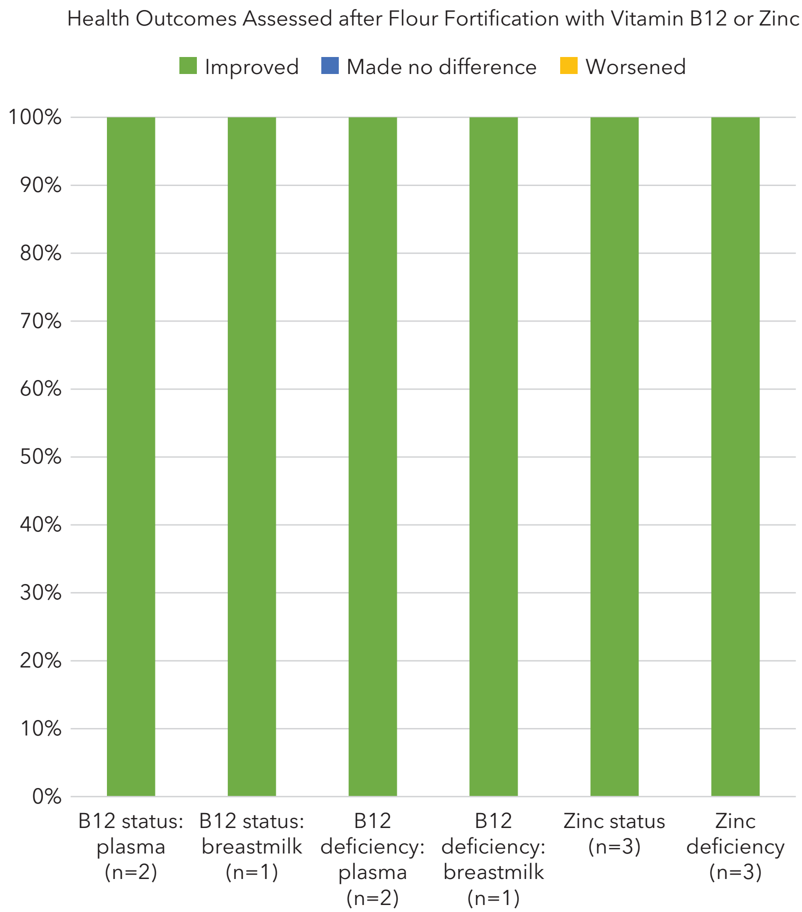

Figure 7 A summary of research on health outcomes assessed after flour fortification with vitamin B12 or zinc: fortification improved, worsened or made no difference in the health outcome. The number in parentheses reflects the number of analyses conducted. If studies reported overall results only, the number reflects the number of studies. If a study reported results by different population groups (e.g. women, children), the number reflects the number of population groups.

- Few analyses showed that health outcomes worsened after fortification. This was the case for cancer, folate deficiency, anemia, hemoglobin and iron-deficiency anemia. That is, the cases of cancer, folate deficiency, anemia and iron-deficiency anemia increased and levels of hemoglobin decreased after fortification.
- Conflicting results for cancer may be explained by the difference in years since fortification was initiated (e.g. increased incidence of colon cancer after fortification is suggested by studies published in the 2000s; the

opposite is observed in studies published in the 2010s) or by the sample size in studies (e.g. increased breast cancer incidence after fortification is observed in studies with sample sizes <2000; studies that observed no difference or a decreased incidence after fortification have sample sizes >2000 and going into the millions).
- Conflicting results for hemoglobin, anemia and iron-deficiency anemia (i.e. some studies show improvements and some show worsening after fortification) may be explained by (1) the existence of non-nutritional causes of anemia which cannot be addressed by fortification, (2) nutritional causes of anemia not addressed by fortification because a limited number of nutrients were added through fortification and (3) levels of nutrients or fortification compounds used in fortification do not follow international guidelines.

Results from other outcomes:

- All remaining outcomes had data from four or fewer analyses: folate-deficiency anemia, homocysteine status, high homocysteine, vitamin B12 deficiency masking, congenital heart disease, coronary heart disease, stroke, myocardial infarction, cognitive function, hypersensitivity, thyroid- and diabetes-related disorders, orofacial clefts, vitamin B12 status, vitamin B12 deficiency, zinc status and zinc deficiency.
- Cautiously, outcomes with two to four analyses suggest the following relationship with fortification.
- Homocysteine status, high homocysteine, stroke death, myocardial infarction, orofacial clefts, vitamin B12 status, vitamin B12 deficiency, zinc status and zinc deficiency trend toward improvement after fortification.
- Vitamin B12 deficiency masking and cognitive function trend towards showing no difference before and after fortification.
- None of the outcomes with two, three or four analyses show a worsening of health after fortification.

6.1 Results from studies in individual countries that assessed health outcomes before and after fortification

'Before and after' studies are those where health outcomes are measured before food fortification is implemented in a country and then after. Here, the before or pre-fortification period is considered the control group for the after or post-fortification period. Health information can be collected on the same individuals, or there can be different individuals in the pre-fortification period and the post-fortification period. What follows are studies conducted in single countries.

6.1.1 Results from studies where the same individuals were measured before and after fortification

Studies where the same individuals were measured before and after fortification in a single country are summarized in Table 4. One example is from Chile with red blood cell folate data from the same women of reproductive age (Hertrampf et al. 2003). Women's blood was taken before initiation of fortification of wheat flour with folic acid and it was taken 12 months after fortification had started. In these women, red blood cell folate levels were 290 $\pm$ 102 nmol/L in the pre-fortification period and increased to 707 $\pm$ 179 nmol/L in the post-fortification period. Red blood cell folate levels increased within 12 months after fortification started, suggesting that fortification of wheat flour with folic acid improved a biological marker of folate status.

Sometimes, pre- and post-fortification studies may not show clear improvements in nutrient status. For example, South Africa experienced an improvement in the nutritional status of one nutrient added through fortification (i.e. folic acid which was further supported by reductions in neural tube defects (Sayed et al. 2008)) but not in another nutrient added through fortification (i.e. iron) (Modjadji et al. 2007). The results from the Modjadji study suggested that an iron compound more bioavailable than the electrolytic iron specified in the country standard could be warranted (UNICEF and Food Fortification Initiative 2004).

6.1.2 Results from studies where different individuals were measured before and after fortification

Studies where different individuals were measured before and after fortification in a single country are summarized in Table 5 for neural tube defects and Table 6 for other health outcomes.

A study from Iran included neural tube defect data collected from different babies (Abdollahi et al. 2011) (Table 5). The researchers reported neural tube defects before fortification of wheat flour with folic acid (years 2006–2007) and after fortification (2007–2008). There were 31.6 and 21.9 neural tube defects per 10 000 live and still births between the time periods, respectively, pointing to a 31% reduction in neural tube defects after fortification of wheat flour with folic acid. Wheat flour fortification with folic acid improved a functional outcome; this was a consistent finding in all countries which studied neural tube defects.

In Cameroon, women and pre-school children's nutritional status was measured before and after initiation of oil fortification with vitamin A and wheat flour fortification with multiple nutrients: folic acid, iron, vitamin B12 and zinc (Engle-Stone et al. 2017) (Table 6). Plasma vitamin B12 levels were higher in women and children in the post-fortification period than in the pre-fortification period; the same was true for breastmilk vitamin B12 levels in lactating

Table 4 Health outcome results from studies where the same individuals were measured before and after wheat flour fortification[a]

Country	Study	Nutrient[b]	Health outcome[c]	Individuals studied	Results
Chile	Hirsch et al. 2002[d]	Folic acid	Serum folate; folate deficiency	Older adults (women and men)	Higher serum folate levels in the post-fortification than pre-fortification period; prevalence of folate deficiency lower in post-fortification than pre-fortification period
Chile	Hertrampf et al. 2003	Folic acid	Serum folate; red blood cell (RBC) folate; low serum folate, low RBC folate	Women of childbearing age	Higher serum and RBC folate levels in the post-fortification than pre-fortification period; prevalence of low serum and RBC folate lower in post-fortification than pre-fortification period
Iran	Sadighi et al. 2008	Iron[e]	Serum ferritin; hemoglobin; low serum ferritin; anemia; iron-deficiency anemia	Women of childbearing age	No difference between the pre- and post-fortification periods for serum ferritin and hemoglobin; no difference between the pre-and post-fortification periods in the prevalence of low serum ferritin, anemia and iron-deficiency anemia
South Africa[a]	Modjadji et al. 2007	Iron	Serum ferritin; low serum ferritin	Women of childbearing age	No difference in serum ferritin levels between pre- and post-fortification periods; no difference in prevalence of low serum ferritin between pre- and post-fortification periods

South Africa[a]	Modjadji et al. 2007	Folic acid	Serum folate; RBC folate; low serum folate; low RBC folate	Women of childbearing age	Higher serum and RBC folate levels in the post-fortification than pre-fortification period; prevalence of low serum and RBC folate lower in post-fortification than pre-fortification period
South Africa[a]	Modjadji et al. 2007	Multiple[f]	Hemoglobin; low hemoglobin	Women of childbearing age	Higher hemoglobin levels in the post-fortification than pre-fortification period; no difference in prevalence of low hemoglobin between pre- and post-fortification periods
USA[a]	Enquobahrie et al. 2012	Folic acid	Serum folate; serum homocysteine	Adolescents	Higher serum folate and homocysteine[g] levels in the post-fortification than pre-fortification period
USA[a]	Stolzenberg-Solomon et al. 2006; Lin et al. 2008; Stevens et al. 2010; Houghton et al. 2019a,b	Folic acid	Breast cancer	Women 30–55 years (Houghton et al. 2019a), 32–53 years (Houghton et al. 2019b), 45 years or older (Lin et al. 2008), 50–74 years (Stevens et al. 2010), 55–74 years (Stolzenberg-Solomon et al. 2006)	Higher breast cancer incidence in the post-fortification than pre-fortification period (Lin et al. 2008; Houghton et al. 2019b);[h] no difference in breast cancer incidence between pre- and post-fortification periods (Stolzenberg-Solomon et al. 2006; Stevens et al. 2010; Houghton et al. 2019a)

(*Continued*)

Table 4 Health outcome results from studies where the same individuals were measured before and after wheat flour fortification[a] (*Continued*)

Country	Study	Nutrient[b]	Health outcome[c]	Individuals studied	Results
USA[a]	Gibson et al. 2011	Folate[i]	Colorectal cancer incidence	Adults 50–71 years	Lower colorectal cancer incidence with higher folate intake in the post-fortification period, this trend was not observed in the pre-fortification period

RBC, red blood cell

[a] Wheat flour was the only grain fortified with one or more nutrients in most countries. Wheat and maize flour was fortified with nutrients in South Africa and the USA (in addition to rice); the independent effect of any one of these fortified foods on health outcomes cannot be discerned with this study.

[b] Nutrient added to wheat flour through fortification which is purported to affect the health outcome.

[c] Some of the health outcomes measure the concentration of nutrients or other constituents in the blood or breastmilk and some refer to the prevalence (i.e. percentage of people who have the condition), the incidence (i.e. number of people newly diagnosed with the condition), or deaths (i.e. number of people who die due to the condition).

[d] Even though the title of this article refers to masking of vitamin B12 deficiency, the authors presented no data that could support or refute masking of B12 deficiency by fortification with folic acid (Hirsch et al. 2002).

[e] Wheat flour in Iran is fortified with two nutrients that contribute to hemoglobin synthesis: iron and folic acid (Sadighi et al. 2008).

[f] As reported in the article, South Africa fortified wheat and maize flour with folic acid, iron, vitamin A, thiamin, riboflavin, niacin, pyridoxine and zinc; many of which can contribute to hemoglobin synthesis.

[g] The homocysteine results are the opposite of what is expected. When folate levels are adequate, homocysteine levels are usually low (Institute of Medicine 1998). The authors suggest that in adolescence, there is a trend toward an increase in homocysteine levels which was not affected by fortification (Enquobahrie et al. 2012).

[h] This result is the opposite of what is expected.

[i] Folate from any source: foods naturally rich in folate, supplements containing folic acid and folic acid-fortified food (Gibson et al. 2011).

Table 5 Results for neural tube defects as the health outcome from studies where different individuals were measured before and after wheat flour fortification[a]

Country	Study	Nutrient[b]	Health outcome[c]	Individuals studied	Results
Argentina	López-Camelo et al. 2010; Bidondo et al. 2015; Sargiotto et al. 2015	Folic acid	Neural tube defects	Live births and still births weighing 500 g or more	Prevalence of NTDs lower in post-fortification than pre-fortification period
Australia	Botto et al. 2006; Hilder 2016	Folic acid	Neural tube defects	Live births, still births and terminations	Prevalence of NTDs lower in post-fortification than pre-fortification period[d]
Brazil[a]	Pacheco et al. 2009; López-Camelo et al. 2010; Pacheco Santos et al. 2016	Folic acid	Neural tube defects	Live births (all studies) and still births weighing 500 g or more (López-Camelo et al. 2010; Pacheco Santos et al. 2016)	Prevalence of two types of NTDs lower in post-fortification than pre-fortification period: anencephaly, cephalocele (López-Camelo et al. 2010); no difference in prevalence of one type of NTD between the pre- and post-fortification periods: total spina bifida (López-Camelo et al. 2010); no difference in NTD prevalence between post-fortification and pre-fortification periods (Pacheco et al. 2009); prevalence of NTDs lower in post-fortification than pre-fortification period (Pacheco Santos et al. 2016)
Canada	Ray et al. 2002; Botto et al. 2006	Folic acid	Neural tube defects	Live births, still births and terminations	Prevalence of NTDs lower in post-fortification than pre-fortification period

(*Continued*)

Table 5 Results for neural tube defects as the health outcome from studies where different individuals were measured before and after wheat flour fortification[a] (*Continued*)

Country	Study	Nutrient[b]	Health outcome[c]	Individuals studied	Results
Chile	Castilla et al. 2003; López-Camelo et al. 2005, 2010; Corral et al. 2006; Nazer et al. 2007; Cortés et al. 2012; Nazer and Cifuentes 2013	Folic acid	Neural tube defects	Live births (all studies) and still births weighing 500 g or more (Castilla et al. 2003; López-Camelo et al. 2005, 2010; Corral et al. 2006; Nazer et al. 2007; Cortés et al. 2012; Nazer and Cifuentes 2013)	Prevalence of NTDs lower in post-fortification than pre-fortification period
Costa Rica[a]	Tacsan Chen and Ascencio Rivera 2004; Barboza Argüello and Umaña Solís 2011; Barboza Argüello et al. 2015	Folic acid	Neural tube defects	Live births (all studies) and still births weighing 500 g or more (Tacsan Chen and Ascencio Rivera 2004; Barboza Argüello and Umaña Solís 2011; Barboza Argüello et al. 2015)	Prevalence of NTDs lower in post-fortification than pre-fortification period[e]
Iran	Abdollahi et al. 2011	Folic acid	Neural tube defects	Live births and still births weighing 500 g or more with a gestational age of 20 weeks or more	Prevalence of NTDs lower in post-fortification than pre-fortification period

Peru	Ricks et al. 2012; Sanabria Rojas et al. 2013; Tarqui-Mamani et al. 2016	Folic acid	Neural tube defects	Live births and still births (Ricks et al. 2012; Sanabria Rojas et al. 2013); live births (Tarqui-Mamani et al. 2016)	No difference in the prevalence of NTDs between post-fortification and pre-fortification periods (Ricks et al. 2012); prevalence of NTDs lower in post-fortification than pre-fortification period (Sanabria Rojas et al. 2013); prevalence of spina bifida lower in post-fortification than pre-fortification period (Tarqui-Mamani et al. 2016); no difference in the prevalence of anencephaly and encephalocele between post-fortification and pre-fortification periods (Tarqui-Mamani et al. 2016)
South Africa[a]	Sayed et al. 2008	Folic acid	Neural tube defects	Live births and still births	Prevalence of NTDs lower in post-fortification than pre-fortification period
USA[a]	Honein et al. 2001; CDC 2004; Botto et al. 2006	Folic acid	Neural tube defects	Live births (Honein et al. 2001) Live births, still births and terminations (Botto et al. 2006); live births, still births, terminations, and fetal deaths[f] (CDC 2004)	Prevalence of NTDs lower in post-fortification than pre-fortification period

NTDs, neural tube defects

[a] Wheat flour was the only grain fortified with folic acid in most countries. Wheat and maize flour were fortified with folic acid in Brazil, Costa Rica (in addition to rice and milk), South Africa and the USA (in addition to rice); the independent effect of any one of these fortified foods on health outcomes cannot be discerned with this study.

[b] Nutrient added to wheat flour through fortification which is purported to affect the health outcome.

[c] In this table, the health outcome refers to the prevalence (or percentage) of livebirths, still births, fetal deaths and/or terminations affected by a neural tube defect such as spina bifida or anencephaly.

[d] Australia experienced decreases in the prevalence of NTDs when the country went from no fortification to voluntary fortification (Botto et al. 2006) and from voluntary to mandatory fortification (Hilder 2016).

[e] Wheat flour was the first food mandated to be fortified with folic acid in Costa Rica. With the addition of subsequent foods mandated to be fortified with folic acid (maize flour, milk, rice) and with an increase in the folic acid levels in wheat flour (from 1.5 mg/kg to 1.8 mg/kg), NTDs continued to decrease (Barboza Argüello and Umaña Solís 2011).

[f] 'Fetal death refers to the spontaneous intrauterine death of a fetus at any time during pregnancy. Fetal deaths later in pregnancy (at 20 weeks of gestation or more, or 28 weeks or more, for example) are also sometimes referred to as stillbirths.' (CDC 2020).

women. Consistent with these findings, the prevalence of low plasma (women and children) and breastmilk (women) vitamin B12 levels was lower in post-fortification than pre-fortification period. These vitamin B12 results suggest that wheat flour fortification with vitamin B12 improved nutritional outcomes in the country.

Results for the nutritional status of folic acid, zinc and hemoglobin/anemia also suggested that fortification was adequately implemented in Cameroon. For iron, three indicators of nutritional status were measured: plasma ferritin, soluble transferrin receptor and body iron stores. These were used to measure the prevalence of low plasma ferritin and high-soluble transferrin receptors (both markers of iron deficiency); together with hemoglobin, plasma ferritin was used to calculate the prevalence of simultaneous iron deficiency and anemia. Most of these measures pointed toward improvements in iron status for women and children except for the prevalence of low plasma ferritin (women) and iron-deficiency anemia (women and children) which was not different between the pre- and post-fortification periods.

The same study design was used to investigate potential negative health impacts of fortification (Table 6). In one study conducted in the USA, researchers surmised that people with low vitamin B12 deficiency and no anemia who consumed grains (i.e. wheat flour, maize flour and rice) fortified with folic acid could be at risk of developing vitamin B12 deficiency (Qi et al. 2014). In other words, since folic acid is provided through grain fortification in the USA, these individuals will not develop anemia. However, since vitamin B12 is not provided through grain fortification in the USA, they may develop vitamin B12 deficiency. The researchers assessed if the prevalence of older adults with vitamin B12 deficiency and no anemia changed between pre and post folic-acid fortification periods. If folic acid was masking vitamin B12 deficiency, one would expect an increase in the post-fortification period in the prevalence of vitamin B12 deficiency and no anemia in the same adults. There was no change in the prevalence from the pre- to the post-fortification period, suggesting there was no masking of vitamin B12 deficiency by grain fortification with folic acid.

6.2 Results from trend studies in individual countries that assessed health outcomes multiple times after fortification

When information on a health outcome is available for many years after fortification has started, trend studies can be completed (Table 7). For example, Saudi Arabia began voluntary wheat flour fortification with folic acid and other nutrients in 2001 (Safdar et al. 2007). Investigators had information on the number of babies born with neural tube defects for 3 years before fortification started (~15, 30 and 20 per 10 000 births, respectively) and for 5 years after fortification started (~15, 12, 10, 10 and 9 per 10 000 births, respectively). The

Table 6 Results for health outcomes other than neural tube defects from studies where different individuals were measured before and after wheat flour fortification[a]

Country	Study	Nutrient[b]	Health outcome[c]	Individuals studied	Results
Australia	Brown et al. 2011	Folic acid	Serum folate; red blood cell (RBC) folate; low serum folate; low RBC folate	Blood samples analyzed in a public hospital's laboratory	Higher serum folate and RBC folate levels in the post-fortification than pre-fortification period; prevalence of low serum folate and low RBC folate lower in post-fortification than pre-fortification period in women and children
Australia	Beckett et al. 2017	Folic acid	Plasma homocysteine; high homocysteine; serum folate; low serum folate; RBC folate	Women and men 65 years or older	Higher serum folate and RBC folate levels in the post-fortification than pre-fortification period; lower plasma homocysteine[d] levels in the post-fortification than pre-fortification period; prevalence of high homocysteine levels (hyperhomocysteinemia)[d] and low serum folate lower in post-fortification than pre-fortification period
Brazil[a]	Britto et al. 2014	Folic acid	Serum folate; red blood cell folate	Pregnant women, children, adolescents, adults, elderly	Higher serum folate and red blood cell folate levels in the post-fortification than pre-fortification period in all age groups
Brazil[a]	Assunção and Santos 2007; Assunção et al. 2007; Costa et al. 2009; Fujimori et al. 2011, Assunção et al. 2012	Iron	Hemoglobin (Assunção et al. 2007, Fujimori et al. 2011; Assunção et al. 2012); anemia (all studies)	Children less than 6 years (Assunção et al. 2007, 2012; Costa et al. 2009) Pregnant women (Fujimori et al. 2011)	No difference in hemoglobin levels (Assunção et al. 2007); higher hemoglobin levels in post-fortification than pre-fortification period (Fujimori et al. 2011); lower hemoglobin levels in post-fortification than pre-fortification period (Assunção et al. 2012); prevalence of anemia higher in post-fortification than pre-fortification (Assunção et al. 2007, 2012);[e] prevalence of anemia lower in post-fortification than pre-fortification (Costa et al. 2009; Fujimori et al. 2011)[f]

(*Continued*)

Table 6 Results for health outcomes other than neural tube defects from studies where different individuals were measured before and after wheat flour fortification[a] (*Continued*)

Country	Study	Nutrient[b]	Health outcome[c]	Individuals studied	Results
Cameroon	Engle-Stone et al. 2017[g]	Folic acid	Plasma folate; low plasma folate	Women of childbearing age, children 12–59 months	Higher plasma folate levels in the post-fortification than pre-fortification period in women and children; prevalence of low plasma folate lower in post-fortification than pre-fortification period in women and children
Cameroon	Engle-Stone et al. 2017[g]	Iron	Plasma ferritin; soluble transferrin receptor; body iron stores; low plasma ferritin; high soluble transferrin receptor; iron-deficiency anemia	Women of childbearing age	Higher plasma ferritin levels and body iron stores in the post-fortification than pre-fortification period; lower soluble transferrin receptor levels in the post-fortification than pre-fortification period;[h] no difference in prevalence of low plasma ferritin and iron-deficiency anemia between post-fortification and pre-fortification period; prevalence of high-soluble transferrin receptor and low body iron stores lower in post-fortification than pre-fortification period
Cameroon	Engle-Stone et al. 2017[g]	Iron	Plasma ferritin; soluble transferrin receptor; body iron stores; low plasma ferritin; high soluble transferrin receptor; iron-deficiency anemia	Children 12–59 months	Higher plasma ferritin levels and body iron stores in the post-fortification than pre-fortification period; lower soluble transferrin receptor levels in the post-fortification than pre-fortification period;[h] no difference in prevalence of iron-deficiency anemia between post-fortification and pre-fortification period; prevalence of low plasma ferritin lower in post-fortification than pre-fortification period in children; prevalence of high soluble transferrin receptor and low body iron stores lower in post-fortification than pre-fortification period

Cameroon	Engle-Stone et al. 2017[g]	Vitamin B12	Plasma vitamin B12; breastmilk vitamin B12 (women only); prevalence low vitamin B12; prevalence low breastmilk vitamin B12 (women only)	Women of childbearing age, children 12–59 months	Higher plasma (women and children) and breastmilk (women) vitamin B12 levels in the post-fortification than pre-fortification period; prevalence of low plasma (women and children) and breastmilk (women) vitamin B12 lower in post-fortification than pre-fortification period
Cameroon	Engle-Stone et al. 2017[g]	Zinc	Plasma zinc; low plasma zinc	Women of childbearing age, children 12–59 months	Higher plasma zinc levels in the post-fortification than pre-fortification period in women and children; prevalence of low plasma zinc lower in post-fortification than pre-fortification period in women and children
Cameroon	Engle-Stone et al. 2017[g]	Multiple	Hemoglobin; anemia	Women of childbearing age, children 12–59 months	In women and children, no difference in hemoglobin levels between the post-fortification and pre-fortification period; prevalence of anemia lower in post-fortification than pre-fortification period in women, no difference in prevalence of anemia in post-fortification and pre-fortification period in children
Canada	Ray et al. 2003; Liu et al. 2004	Folic acid	Serum folate (Ray et al. 2003; Liu et al. 2004); RBC folate (Liu et al. 2004); plasma homocysteine (Liu et al. 2004); folate deficiency (Ray et al. 2003)	Women 65 years and older (Ray et al. 2003), women 19–44 years and women and men 65 years and older (Liu et al. 2004)	In women and older adults: higher serum and RBC folate levels in the post-fortification than pre-fortification period, lower plasma homocysteine levels in the post-fortification than pre-fortification period; in women 65 years and older: prevalence of folate deficiency lower in post-fortification than pre-fortification period
Canada	Mason et al. 2007	Folic acid	Incidence of colorectal cancer	All ages	Incidence of colorectal cancer higher in post-fortification than pre-fortification period[e]

(*Continued*)

Table 6 Results for health outcomes other than neural tube defects from studies where different individuals were measured before and after wheat flour fortification[a] (*Continued*)

Country	Study	Nutrient[b]	Health outcome[c]	Individuals studied	Results
Chile	Hirsch et al. 2009	Folic acid	Hospital discharge due to colon cancer	Patients discharged from public or private hospitals	Following the secular trend, higher hospital discharges due to colon cancer in the post-fortification than pre-fortification period[i]
Costa Rica[a]	Tacsan Chen and Ascencio Rivera 2004	Folic acid	Serum folate; folate deficiency	Women	In urban and rural areas: higher serum folate levels in the post-fortification than pre-fortification period; in urban and rural areas: prevalence of folate deficiency lower in the post-fortification than pre-fortification period
Costa Rica[a]	Martorell et al. 2015	Iron	Serum ferritin; hemoglobin; iron deficiency; iron-deficiency anemia; anemia	Children 1–7 years	Higher serum ferritin and hemoglobin in the post-fortification than pre-fortification period; prevalence of iron deficiency, iron-deficiency anemia and anemia lower in the post-fortification than pre-fortification period
Costa Rica[a]	Martorell et al. 2015	Vitamins A, B1, B2, B9, B12, E Minerals iron, zinc[f]	Hemoglobin; anemia	Women 15–45 years	Higher hemoglobin levels in the post-fortification than pre-fortification period; prevalence of anemia lower in the post-fortification than pre-fortification period
Fiji	National Food and Nutrition Centre 2012	Folic acid, iron, niacin, riboflavin, thiamin, zinc	Serum ferritin; hemoglobin; serum folate; serum zinc; low serum ferritin; anemia; low serum folate; low serum zinc	Women of childbearing age	Higher serum ferritin, hemoglobin, serum folate and serum zinc levels in the post-fortification than pre-fortification period; prevalence of low serum ferritin, anemia, low serum folate and low serum zinc lower in post-fortification than pre-fortification period

Iran	Abdollahi et al. 2011	Folic acid	Serum folate; plasma homocysteine; low serum folate; high plasma homocysteine	Women of childbearing age	Higher serum folate levels in the post-fortification than pre-fortification period; lower plasma homocysteine levels in the post-fortification than pre-fortification period;[j] prevalence of low serum folate and high plasma homocysteine lower in post-fortification than pre-fortification period
Iran	Sadighi et al. 2009	Iron[k]	Serum ferritin; hemoglobin; low serum ferritin; anemia; iron-deficiency anemia	Women of childbearing age	Higher serum ferritin levels in the post-fortification than pre-fortification period, lower hemoglobin levels[e] in the post-fortification than pre-fortification period; prevalence of low serum ferritin lower in post-fortification than pre-fortification period; prevalence of anemia[e] and iron-deficiency anemia[e] higher in post-fortification than pre-fortification period
USA[a]	CDC 2003; Dietrich et al. 2005; Pfeiffer et al. 2012, 2019	Folic acid	Serum folate (all studies); RBC folate (all studies); low serum folate (Dietrich et al. 2005; Pfeiffer et al. 2012); low RBC folate (Dietrich et al. 2005; Pfeiffer et al. 2012)	Children (Pfeiffer et al. 2012, 2019) Adults (Dietrich et al. 2005; Pfeiffer et al. 2012, 2019) Adult women (CDC 2003)	Higher serum and RBC folate levels in the post-fortification than pre-fortification period; prevalence of low serum folate and low RBC folate lower in post-fortification than pre-fortification period
USA[a]	Mills et al. 2003; Qi et al. 2014	Folic acid	Masking of vitamin B12 deficiency	Older adults with both low vitamin B12 status (or vitamin B12 deficiency) and no anemia (or macrocytosis[lb])	Prevalence of low vitamin B12 status and no anemia the same in the pre- and post-fortification periods; no evidence of masking;[m] prevalence of vitamin B12 deficiency and no anemia (or macrocytosis) the same (or lower) in post-fortification than pre-fortification period; no evidence of masking[m]

(*Continued*)

Table 6 Results for health outcomes other than neural tube defects from studies where different individuals were measured before and after wheat flour fortification[a] (*Continued*)

Country	Study	Nutrient[b]	Health outcome[c]	Individuals studied	Results
USA[a]	Mason et al. 2007	Folic acid	Incidence of colorectal cancer	All ages	Incidence of colorectal cancer higher in post-fortification than pre-fortification period[e]
Venezuela[a]	Layrisse et al. 1996, 2002	Iron[m]	Serum ferritin (both studies); low serum ferritin (both studies); anemia (both studies); iron-deficiency anemia (Layrisse et al. 1996)	Children and adolescents	Higher serum ferritin levels in the post-fortification than pre-fortification period (both studies); prevalence of low serum ferritin and anemia lower in post-fortification than pre-fortification period (Layrisse et al. 1996); no difference in prevalence of low serum ferritin or anemia in post-fortification and pre-fortification period (Layrisse et al. 2002); no difference in prevalence of iron-deficiency anemia in post-fortification and pre-fortification period (Layrisse et al. 1996)

NTDs, neural tube defects; RBC, red blood cell

[a] Wheat flour was the only grain fortified with one or more nutrients in most countries. Wheat and maize flour were fortified with nutrients in Brazil, Costa Rica (in addition to rice and milk), the USA (in addition to rice) and Venezuela; the independent effect of any one of these fortified foods on health outcomes cannot be discerned with this study.

[b] Nutrient added to wheat flour through fortification which is purported to affect the health outcome.

[c] Some of the health outcomes measure the concentration of nutrients or other constituents in the blood or breastmilk and some refer to the prevalence (i.e. percentage of people who have the condition), the incidence (i.e. number of people newly diagnosed with the condition), or deaths (i.e. number of people who die due to the condition).

[d] 'Plasma homocysteine concentration increases when inadequate quantities of folate are available to donate the methyl group that is required to convert homocysteine to methionine' (Institute of Medicine 1998). When folate levels are high, homocysteine levels are usually low and the prevalence of people with high homocysteine levels (e.g. hyperhomocysteinemia) are usually low.

[e] This result is the opposite of what is expected.

[f] The post-fortification prevalence of anemia was compared to the pre-fortification prevalence of anemia for preschool children (1) in the same southeast region of the country as well as (2) other regions of the country. The results were the same.

[g] For this study from Cameroon, adjusted results are presented (not unadjusted results).

[h] A decrease in soluble transferrin receptor levels reflect an improvement in iron status.

[i] The upward trend in Chile mirrors the increase in deaths due to colon cancer evident from 1990 to 2003 (Donoso et al. 2006) – preceding the introduction of fortification with folic acid in the country in 2000.
[j] A decrease in homocysteine levels reflects an improvement in folate status.
[k] Wheat flour in Iran is fortified with two nutrients that contribute to hemoglobin synthesis: iron and folic acid (Sadighi et al. 2008).
[l] Macrocytosis means enlarged red blood cells.
[m] If the prevalence of individuals with vitamin B12 deficiency (or low vitamin B12 status) and no anemia (or no macrocytosis) increased between the pre and post folic-acid fortification periods, that would be evidence of fortification with folic acid masking of vitamin B12 deficiency.
[n] Wheat and maize flour in Venezuela are fortified with other nutrients that contribute to hemoglobin synthesis: iron, vitamin A, thiamin and riboflavin (Layrisse et al. 1996).

60% reduction in neural tube defects from pre- to post fortification periods suggests that wheat flour fortification with folic acid improved a functional outcome.

The same study design was used to investigate potential negative health impacts of fortified food, such as cancer. Vollset et al. (2013) collated the number of colorectal deaths per 100 000 population in the USA from 1950 to 2010. In the country, voluntary fortification of breakfast cereals with folic acid began in 1973 and mandatory fortification of grains with folic acid became effective in 1998. If fortification with folic acid accelerates death from cancer, cancer deaths from 1973 (or 1998) until 2010 should increase. The data show the opposite trend for women and men: during this 60-year period, there was a decline in colorectal cancer deaths. These results suggest that fortification with folic acid does not cause cancer deaths.

6.3 Results from cross-sectional studies in individual countries that assessed health outcomes and fortification exposure simultaneously

Cross-sectional studies are another type of design that can inform the health impact of a fortification program. Cross-sectional means that information was collected at one point of time only. These studies are especially useful in cases where no pre-fortification information is available, so it is not possible to complete a before-and-after study. Three countries have completed such studies (Table 8).

One example is from Oman where a one-time, cross-sectional survey was conducted in 2004 (Grimm et al. 2012). Ferritin and C-reactive protein were assessed in non-pregnant women of childbearing age. This information was used to calculate the percentage of women with iron deficiency, a biomarker of iron status. Families were asked how much wheat flour they consumed in the previous two months and the total number of individuals living or working in the household during this time. Additionally, wheat flour samples were taken from homes and analyzed for the presence of fortificant iron. This information was used to calculate the monthly per capita consumption of fortified wheat flour.

The researchers then completed a dose-response analysis and found that the prevalence of iron deficiency was lowest in women whose households consumed the highest amount of fortified wheat flour: 26.8% compared with 38.8%. These results are in the direction one would expect if flour fortified with iron is being produced and consumed in the country. While the study design does not allow one to conclude that fortification caused a reduction in iron deficiency, the results suggest that fortification is contributing to improving the iron status of women in Oman.

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Table 7 Results from trend studies where health outcomes were measured multiple times after wheat flour fortification began[a]

Country	Study	Nutrient[b]	Health outcome[c]	Individuals studied	Results
Canada	Persad et al. 2002; De Wals et al. 2003; Liu et al. 2004; De Wals et al. 2007	Folic acid	Neural tube defects	Live births, still births and terminations	Prevalence of NTDs lower in post-fortification than pre-fortification period
Canada	French et al. 2003; Grupp et al. 2011	Folic acid	Pediatric cancers	Children less than 17 years (French et al. 2003) Children less than 4 years (Grupp et al. 2011)	Prevalence of neuroblastoma and Wilms' tumor lower in post-fortification than pre-fortification period; no difference in the prevalence of acute lymphoblastic leukemia, hepatoblastoma, embryonal cancers or brain cancers between post- and pre-fortification periods
Canada	Ionescu-Ittu et al. 2009	Folic acid	Congenital heart disease	Live births, still births	Prevalence of congenital heart disease[d] lower in post-fortification than pre-fortification period
Canada	Yang et al. 2006	Folic acid	Stroke death	Adults 40 years of age and older	Decrease in deaths from stroke was greater in the post-fortification period (−5.4% annually) than pre-fortification period (−1.0% annually)
Jordan	Amarin and Obeidat 2010	Folic acid	Neural tube defects	Live births	Prevalence of NTDs lower in post-fortification than pre-fortification period
Oman	Alasfoor et al. 2010	Folic acid	Spina bifida[e]; non-spina bifida neural tube defects[f]	Live births	Prevalence of spina bifida lower in post-fortification than pre-fortification period; no difference in the prevalence of other, non-spina-bifida NTDs between post- and pre-fortification periods

(*Continued*)

Table 7 Results from trend studies where health outcomes were measured multiple times after wheat flour fortification began[a] (*Continued*)

Country	Study	Nutrient[b]	Health outcome[c]	Individuals studied	Results
Saudi Arabia	Safdar et al. 2007	Folic acid	Neural tube defects	Live births	Prevalence of NTDs lower in post-fortification than pre-fortification period
Tanzania[a]	Noor et al. 2017	Folic acid	Plasma folate; folate deficiency	Women	Higher plasma folate levels in the post-fortification than pre-fortification period; prevalence of folate deficiency lower in post-fortification than pre-fortification period
USA[a]	Vollset et al. 2013; Keum and Giovannucci 2014; Siegel et al. 2019	Folic acid	Colorectal cancer incidence (Keum and Giovannucci 2014; Siegel et al. 2019) and mortality (Vollset et al. 2013; Keum and Giovannucci 2014; Siegel et al. 2019); breast cancer incidence and mortality (Siegel et al. 2019)	All ages and sexes (Siegel et al. 2019) Adults (Vollset et al. 2013; Keum and Giovannucci 2014)	Incidence of colorectal cancer decreased for women (from 1998 to 2008), men (from 1998 to 2015) (Siegel et al. 2019) and all adults (from 1975 to 2009) (Keum and Giovannucci 2014); incidence of breast cancer decreased for women (from 1999 to 2004) (Siegel et al. 2019); death from colorectal cancer decreased in women and men (from 1950 to 2010) (Vollset et al. 2013); death from colorectal cancer decreased in black and white women and men (from 1975 to 2009) (Keum and Giovannucci 2014); death from colorectal cancer decreased in women (from 1975 to 2016) and men (from 1987 to 2016) (Siegel et al. 2019); death from breast cancer decreased in women (from 1990 to 2016) (Siegel et al. 2019)

USA[a]	Mathews et al. 2002; Williams et al. 2002, 2005, 2015	Folic acid	Neural tube defects	Live births (all studies), still births (Williams et al. 2002, 2015), fetal deaths[g] (Williams et al. 2002, 2005) and terminations (Williams et al. 2002, 2005, 2015)	Prevalence of NTDs lower in post-fortification than pre-fortification period (Mathews et al. 2002; Williams et al. 2002); for Hispanic and non-Hispanic whites: prevalence of NTDs lower in post-fortification than pre-fortification period (Williams et al. 2005); for non-Hispanic blacks: no difference in prevalence of NTDs between post-fortification and pre-fortification periods (Williams et al. 2005)
USA[a]	Yang et al. 2006	Folic acid	Stroke death	Adults 40 years of age and older	Decrease in deaths from stroke was greater in the post-fortification period (−2.9% annually) than pre-fortification period (−0.3% annually)

[a] Wheat flour was the only grain fortified with folic acid in most countries. Wheat and maize flour were fortified with folic acid in Tanzania and the USA (in addition to rice); the independent effect of any one of these fortified foods on health outcomes cannot be discerned with this study.
[b] Nutrient added to wheat flour through fortification which is purported to affect the health outcome.
[c] Some of the health outcomes measure the concentration of nutrients or other constituents in the blood or breastmilk and some refer to the prevalence (i.e. percentage of people who have the condition), the incidence (i.e. number of people newly diagnosed with the condition), or deaths (i.e. number of people who die due to the condition).
[d] Severe congenital heart defects studied were as follows: 'tetralogy of Fallot, endocardial cushion defects, univentricular hearts, truncus arteriosus, or transposition complexes' (Ionescu-Ittu et al. 2009).
[e] Spina bifida is a type of neural tube defect.
[f] The other neural tube defects that were not spina bifida were not specified (Alasfoor et al. 2010).
[g] 'Fetal death refers to the spontaneous intrauterine death of a fetus at any time during pregnancy. Fetal deaths later in pregnancy (at 20 weeks of gestation or more, or 28 weeks or more, for example) are also sometimes referred to as stillbirths.' (CDC 2020).

Table 8 Results from cross-sectional studies in individual countries that assessed health outcomes and fortification exposure simultaneously.

Country	Study	Nutrient[a]	Health outcome[b]	Individuals studied	Results
Oman	Grimm et al. 2012	Iron	Iron deficiency	Women of childbearing age	Prevalence of iron deficiency lower in women whose households consumed the most fortified wheat flour compared with households who purchased the least fortified flour
Colombia	Fothergill et al. 2019	Iron	Low serum ferritin	Women of childbearing age Children 2–4 and 5–12 years	Prevalence of low serum ferritin did not differ by intake of wheat flour-containing foods
Colombia	Fothergill et al. 2019	Folic acid, iron, riboflavin, thiamin	Anemia	Women of childbearing age Children 2–4 and 5–12 years	Prevalence of anemia was lowest in children 2–4 years who were in the highest quartile for intake of wheat flour-containing foods; prevalence of anemia did not differ by intake of wheat flour-containing foods[c] for women and children 5–12 years
Uzbekistan	Hund et al. 2013[d]	Iron	Iron depletion[e]	Women of childbearing age	Prevalence of iron depletion did not differ by household possession of flour that should be fortified[f] and bread that should be fortified
Uzbekistan	Hund et al. 2013[d]	Folic acid	Folate deficiency	Women of childbearing age	Prevalence of folate deficiency was higher in households possessing flour that should be fortified;[f,g] prevalence of folate deficiency was lower in households possessing bread that should be fortified

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Uzbekistan	Hund et al. 2013[d]	Folic acid, iron, riboflavin, thiamin, zinc	Anemia	Women of childbearing age	Prevalence of anemia did not differ by household possession of flour that should be fortified[f] and bread that should be fortified

[a] Nutrient added to wheat flour through fortification which is purported to affect the health outcome.
[b] Some of the health outcomes measure the concentration of nutrients or other constituents in the blood or breastmilk and some refer to the prevalence (i.e. percentage of people who have the condition), the incidence (i.e. number of people newly diagnosed with the condition), or deaths (i.e. number of people who die due to the condition).
[c] Wheat-flour containing foods includes foods such as bread, pasta and cookies; the fortification status of the flour used to make these foods could not be confirmed (Fothergill et al. 2019).
[d] The unpublished report by Northrop-Clewes et al. (2013) contains the same information as this published article.
[e] Serum ferritin <12 μg/L; in summarizing the results in this chapter, this health outcome was classified as iron deficiency.
[f] While the fortification status of household wheat flour was measured (41.6% of flour was fortified), the researchers did not compare iron depletion, folate deficiency or anemia prevalence between households with fortified flour and those with non-fortified flour.
[g] This result is the opposite of what is expected.

6.4 Other relevant evidence from individual countries

6.4.1 Result from cost-effectiveness studies in individual countries

Economists compare the costs of operating programs, such as fortification, with the effectiveness of such programs. They do this at two time points: before a program has started using hypothesized costs and outcomes (e.g. Dalziel et al. 2009), and after a program has operated using actual costs and outcomes. The latter studies are described here.

After fortification initiation, three countries compared the costs of adding folic acid to flour, the costs of treating people with spina bifida, a type of neural tube defect, and the effectiveness of fortification in reducing neural tube defects (Table 9). Each study showed significant annual net savings in healthcare expenses when spina bifida is prevented through fortification: 2.0–2.6 million international dollars in Chile (Llanos et al. 2007), 40.6 million Rand in South Africa (Sayed et al. 2008) and 88–603 million US dollars in the USA (Grosse et al. 2005, 2016). Since these are annual figures, every year of fortification leads to these net savings.

6.4.2 Results from cross-sectional studies in individual countries that assessed health outcomes only in the post-fortification period

As noted earlier, some researchers publish only post-fortification results. These are rarely informative without a comparison to pre-fortification values. Table 10 lists studies highlighting an outcome for which there are no pre- and post-fortification results: folate deficiency for Canada and folate-deficiency anemia for the USA. In both cases, the post-fortification prevalence of these outcomes is <1% suggesting that fortification with folic is contributing to keeping these values low.

6.4.3 Results from modeling the health impact of wheat flour fortification in individual countries

With information from singles countries, it is possible to statistically model the health impact that fortification is having (Table 11). Tice et al. (2001) modeled the impact of mandatory fortification of wheat flour, maize flour and rice with folic acid on myocardial infarctions (heart attacks) and death from coronary heart disease (CHD) in the USA. Using conservative assumptions of how much fortification would reduce homocysteine levels (i.e. by 5 μmol/L) and what those reductions would be due to the risk of coronary heart disease (i.e. decrease by 9%), they estimated that up to 1% of heart attacks and deaths from CHD could

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Table 9 Results from cost-effectiveness studies in individual countries that assessed costs and health outcomes in the post-fortification period[a]

Country	Study	Nutrient[b]	Health outcome[c]	Individuals studied	Results
Chile	Llanos et al. 2007	Folic acid	Spina bifida[d]	Live births and fetal deaths[e] weighing 500 g or more	Annual net savings in healthcare expenses when spina bifida is prevented through fortification: 2.0–2.6 million international dollars
South Africa[a]	Sayed et al. 2008	Folic acid	Spina bifida[d]	Live births and still births	Annual net savings in healthcare expenses when spina bifida is prevented through fortification: 40.6 million Rand
USA[a]	Grosse et al. 2005, 2016	Folic acid	Spina bifida[d]	Live births	Annual net savings in healthcare expenses when spina bifida is prevented through fortification: 88–145 million US dollars (Grosse et al. 2005) and 299–603 million US dollars (Grosse et al. 2016)
USA[a]	Bentley et al. 2009	Folic acid	Neural tube defects (NTDs); myocardial infarctions (MIs); colon cancer incidence; vitamin B12 masking	Adults 15 years or older	Annual net savings through fortification at current levels (140 μg/100 g) when NTDs, MIs and colon cancer are averted and when masking of B12 deficiency occurs: 780.5 million US dollars

[a] Wheat flour was the only grain fortified with folic acid in Chile. Wheat and maize flour were fortified with folic acid in South Africa and the USA (in addition to rice); the independent effect of any one of these fortified foods on health outcomes cannot be discerned with this study.
[b] Nutrient added to wheat flour through fortification which is purported to affect the health outcome.
[c] Some of the health outcomes measure the concentration of nutrients or other constituents in the blood or breastmilk and some refer to the prevalence (i.e. percentage of people who have the condition), the incidence (i.e. number of people newly diagnosed with the condition), or deaths (i.e. number of people who die due to the condition).
[d] Spina bifida is a type of neural tube defect.
[e] 'Fetal death refers to the spontaneous intrauterine death of a fetus at any time during pregnancy. Fetal deaths later in pregnancy (at 20 weeks of gestation or more, or 28 weeks or more, for example) are also sometimes referred to as stillbirths' (CDC 2020).

Table 10 Results from cross-sectional studies in individual countries that assessed health outcomes in the post-fortification period only[a]

Country	Study	Nutrient[a]	Health outcome[b]	Individuals studied	Results
Canada	Colapinto et al. 2011	Folic acid	Folate deficiency	Females and males 6–79 years	Prevalence of folate deficiency in the post-fortification period was <1%
USA[a]	Odewole et al. 2013	Folic acid	Folate deficiency, folate-deficiency anemia	Adults 50 years and older	Prevalence of folate deficiency in the post-fortification period was 0.1%, prevalence of folate-deficiency anemia in the post-fortification period was 0.1%

[a] Wheat flour was the only grain fortified with folic acid in Canada. Wheat and maize flour and rice were fortified with folic acid in the USA; the independent effect of any one of these fortified foods on health outcomes cannot be discerned with this study.
[b] Nutrient added to wheat flour through fortification which is purported to affect the health outcome.
[c] Some of the health outcomes measure the concentration of nutrients or other constituents in the blood or breastmilk and some refer to the prevalence (i.e. percentage of people who have the condition), the incidence (i.e. number of people newly diagnosed with the condition), or deaths (i.e. number of people who die due to the condition).

be prevented over a 10-year period. Using less-conservative assumptions (i.e. homocysteine levels reduced by 11 μmol/L and CHD reduced by 29%), they estimated that up to 13% of heart attacks and deaths from CHD could be prevented over a 10-year period.

Table 11 Results from modeling the health impact of wheat flour fortification for individual countries[a]

Study	Country	Nutrient[b]	Health outcome[c]	Individuals studied	Results
Tice et al. 2001	USA[a]	Folic acid	Myocardial infarction incidence (MIs); coronary heart disease (CHD) deaths	Adults	Cereal grain fortification could reduce MI incidence and CHD deaths by 1–13% over a 10-year period

[a] In the United States, cereal grains that must be fortified with folic acid include wheat flour, maize flour and rice (Global Fortification Data Exchange 2020d). The independent effect of any one of these fortified foods on health outcomes cannot be discerned with this study.
[b] Nutrient added to wheat flour through fortification which is purported to affect the health outcome.
[c] Some of the health outcomes measure the concentration of nutrients or other constituents in the blood or breastmilk and some refer to the prevalence (i.e. percentage of people who have the condition), the incidence (i.e. number of people newly diagnosed with the condition), or deaths (i.e. number of people who die due to the condition).

6.5 *Results from systematic reviews of multiple studies from multiple countries*

Systematic reviews of the literature compare and contrast the health outcomes reported from multiple studies which can come from the same country but also often have information from different countries. Systematic reviews focused on wheat flour fortification or that include wheat flour fortification are summarized in Table 12.

One systematic review assessed the impact of flour fortification with folic acid on neural tube defects (Castillo-Lancellotti et al. 2013). Twenty seven studies were obtained from nine countries, of which most only fortified wheat flour with folic acid (Chile, Argentina, Canada, Iran, Jordan) and some fortified multiple foods with folic acid (Brazil and South Africa – wheat and maize flour; Costa Rica – wheat and maize flour, rice, milk; USA – wheat flour, maize flour, rice). The authors concluded that 'Fortification of flour with folic acid has had a major impact on [neural tube defects] in all countries where this has been reported.'

The review by van Gool et al. (2018) was difficult to interpret. The document reviewed evidence linking folic acid (from any source, including fortified food) to positive outcomes such as decreasing neural tube defects and to negative outcomes such as masking vitamin B12 deficiency. There was no succinct summary of each of the outcomes. Instead the authors concluded 'the risks carried by a high daily intake of folate equivalents do not outweigh the benefits of folic acid fortification of staple foods, as long as concentrations of serum un-metabolized folic acid, RBC folate, and serum vitamin B12 can be monitored periodically'.

6.6 *Results from meta-analyses of multiple studies from multiple countries*

Meta-analyses go one step further from systematic reviews and take numeric results from multiple studies and re-analyze them, to come up with a new estimate of what the relationship is between fortification and the health outcome. As with systematic reviews, meta-analyses are completed with data from multiple studies and they can be from the same country, or, more often than not, from different countries (Table 13).

Keats et al. (2019) published a meta-analysis of 17 studies of which 16 evaluated a national fortification program. In all cases, wheat and maize flour were fortified with folic acid and the fortification took place for 1–11 years in study countries. Several health outcomes were analyzed: serum folate, folate deficiency and neural tube defects. In eight studies with 6765 women, serum folate increased by 11.94 nmol/L from the pre- to the post-fortification period. In

Table 12 Results from systematic reviews of multiple studies from multiple countries that evaluated the health impact of wheat flour fortification

Study	Countries (n)	Nutrient[a]	Health outcome[b]	Individuals studied	Results
Assunção and Santos 2007	6	Iron	Anemia	Children	Prevalence of anemia lower in post-fortification than pre-fortification period
Castillo-Lancellotti et al. 2013	9	Folic acid	Neural tube defects (NTDs)	Differed depending on article reviewed	Prevalence of NTDs lower in post-fortification than pre-fortification period
Pacheco Santos and Zanon Pereira 2007	5	Folic acid	Neural tube defects	Live births	Prevalence of NTDs lower in post-fortification than pre-fortification period
Pachón et al. 2015	13	Multiple	Iron deficiency, anemia	Children and women	Prevalence of low ferritin lower in post-fortification than pre-fortification period for women; no difference in the prevalence of low ferritin in post-fortification and pre-fortification period in children; no difference in the prevalence of anemia in post-fortification and pre-fortification period in women and children
Rosenthal et al. 2014	15	Folic acid	Neural tube defects	Live births	Prevalence of NTDs lower in post-fortification than pre-fortification period
van Gool et al. 2018	Not specified	Folic acid	Multiple	Multiple	Not clear
van Gool et al. 2020	Not specified	Folic acid	Cognitive function	Not specified	'The hypothesis that cognitive impairment in "subclinical" cyanocobalamin deficiency is folate-mediated is untenable.'

[a] Nutrient added to wheat flour through fortification which is purported to affect the health outcome.
[b] Some of the health outcomes measure the concentration of nutrients or other constituents in the blood or breastmilk and some refer to the prevalence (i.e. percentage of people who have the condition), the incidence (i.e. number of people newly diagnosed with the condition), or deaths (i.e. number of people who die due to the condition).

four studies with 4645 women, the prevalence of women with folate deficiency decreased by 80% from the pre- to the post-fortification period. From eight studies with 19 million data points, the odds of a baby being born with neural tube defects declined by 41% between the pre- and the post-fortification period.

The National Toxicology Program (2015) reviewed research assessing adverse health outcomes after consuming high levels of folate (whether from food sources, supplements containing folic acid or foods fortified with folic acid). Four health outcomes were considered high priority (cancer, cognition, hypersensitivity, and thyroid- and diabetes-related disorders) and thus meta-analyses were conducted. In summary, 96% of 27 million data points showed no relationship between high folate and cancer, and for cognition, hypersensitivity (such as asthma) and thyroid- and diabetes-related disorders, results were 'not supportive' or 'inconclusive' of a relationship between these outcomes and high folate (Table 13). The effect of wheat flour fortification with folic acid cannot be isolated from these studies; however, these results suggest that high folate levels (independent of the source) are not associated with negative health outcomes.

For other health outcomes, that were deemed lower priority by the National Toxicology Program, research results were briefly summarized in their 2015 report. From these summaries, it is not clear what the folate source(s) were. Nevertheless, they consistently found no relationship between folate intake from any source and adverse health outcomes, as follows:

- Cardiovascular outcomes. 'None of the 39 identified meta-analyses reported any adverse effects associated with folic acid intake.'
- Twinning and multiple births. 'While it may be biologically plausible that periconceptional vitamin use plays a role in the incidence of multiple births, the available evidence has been well explored – the most recent human study identified was published in 2006 – so this was not determined to be a high priority topic for this review.'
- Autism. 'Due to weaknesses in the design of studies reporting adverse effects, the currently available literature did not support consideration of autism as a high priority outcome for this review.'
- Other neurological outcomes. 'None of the 10 identified meta-analyses reported adverse effects of folic acid.'
- Other immunological outcomes. 'The majority of other immunological outcomes which were not considered hypersensitive-related, such as autoimmune diseases, did not suggest any adverse effects of folic acid and were not considered a high priority category.'
- Other endocrine and metabolic disease outcomes. 'Body weight, body composition, and BMI constituted the largest group of studies (~50),

Table 13 Results from meta-analyses of multiple before and after studies from multiple countries that evaluated the health impact of wheat flour fortification

Study	Countries or studies (n)	Nutrient[a]	Health outcome[b]	Individuals studied	Results
Atta et al. 2016	52 countries	Folic acid	Spina bifida[c]	Live births; live births and still births; live births, still births and terminations	Prevalence of spina bifida lower in countries with mandatory fortification than voluntary fortification
Blencowe et al. 2010	6 countries	Folic acid	Neural tube defects	Not specified	Prevalence of NTDs lower in post-fortification than pre-fortification period
Das et al. 2013	10 countries	Folic acid	Serum folate; neural tube defects	Women of reproductive age	No difference in serum folate levels between pre- and post-fortification periods; prevalence of NTDs lower in post-fortification than pre-fortification period
Keats et al. 2019	12[d] countries	Folic acid	Serum folate; folate deficiency; neural tube defects	Live births; still births	Higher serum folate levels in the post-fortification than pre-fortification period; prevalence of folate deficiency and NTDs lower in post-fortification than pre-fortification period
Millacura et al. 2017	9 countries	Folic acid	Orofacial clefts	Total births	Prevalence of non-syndromic cleft lip with or without cleft palate is lower in post-fortification than pre-fortification period; no difference in total orofacial clefts, cleft lip with or without cleft palate, cleft palate, non-syndromic orofacial clefts, and non-syndromic cleft palate between pre- and post-fortification periods

National Toxicology Program 2015	43 pooled and meta-analysis studies	Folate[e]	Cancer	Not specified	From 27 million data points, 96% showed no relationship or a protective relationship between high folate (intake or blood levels) and cancer and 4% showed a harmful relationship between high folate (intake or blood levels) and cancer
National Toxicology Program 2015	28 studies and 2 meta-analyses[f]	Folate[e]	'Cognition in conjunction with vitamin B12 deficiency'	Adults	Results 'not supportive' of a relationship between high folate and cognitive impairment in the presence of vitamin B12 deficiency
National Toxicology Program 2015	42 studies and 1 meta-analysis[f]	Folate[e]	Hypersensitivity (e.g. asthma, eczema)	All age groups	Results 'not supportive' or 'inconclusive' of a relationship between high folate and hypersensitivity
National Toxicology Program 2015	72 studies and 1 meta-analysis[f]	Folate[e]	Thyroid- and diabetes-related disorders (e.g. insulin resistance, metabolic syndrome)		Results "not supportive" of a relationship between high folate and thyroid or diabetes-related disorders

[a] Nutrient added to wheat flour through fortification which is purported to affect the health outcome.
[b] Some of the health outcomes measure the concentration of nutrients or other constituents in the blood or breastmilk and some refer to the prevalence (i.e. percentage of people who have the condition), the incidence (i.e. number of people newly diagnosed with the condition), or deaths (i.e. number of people who die due to the condition).
[c] Spina bifida is a type of neural tube defect.
[d] Studies from these 12 countries did not all report on the three outcomes: serum folate, folate deficiency and neural tube defects.
[e] Researchers assessed high levels of folate from any source: non-fortified food, supplements containing folic acid or foods fortified with folic acid; the effect of wheat flour fortification with folic acid cannot be isolated from this study (National Toxicology Program 2015).
[f] Unable to download the meta-analysis results from the study website (https://hawcproject.org/assessment/67/downloads/; https://hawcproject.org/assessment/48/downloads/) (National Toxicology Program 2015).

with only 2 studies reporting any significant relationship between higher folate intake or level and increased body weight. No studies of folate and polycystic ovary syndrome or pancreatitis reported any adverse associations.'
- Other reproductive outcomes. 'None of the 9 meta-analyses reported an adverse effect of folic acid, so reproductive effects were not considered a high priority category.'
- Mortality. '18 meta-analyses have been conducted for several mortality outcomes with a sufficient number of available studies (e.g. all-cause, cardiovascular, cancer, perinatal) and none report any statistically significant adverse meta-estimates.'

6.7 Results from modeling the health impact of wheat flour fortification for multiple countries

With information from multiple countries, it is possible to model fortification's health impact (Table 14). For example, researchers estimated how much of the neural tube defects that can be prevented with folic acid is being prevented through fortification of wheat and/or maize flour with folic acid (Kancherla et al. 2018). The investigators modeled the impact of fortification on three groups of countries: those with high prevention potential, because they have fortification programs in place; those with no prevention potential because they have no fortification programs; and those with modest prevention potential because their fortification programs do not have high coverage or high-enough levels of folic acid to prevent neural tube defects. They estimated 50 270 birth defects were prevented in 2017 where flour was fortified with folic acid.

6.8 Studies from multiple countries that did not assess the independent contribution of mandatorily fortified wheat flour

Additional systematic reviews and meta-analyses were completed that analyzed the health impact of many fortified foods simultaneously, including wheat flour. However, they were not included in this chapter for one of two reasons (Table 15). One, the wheat flour was not fortified as part of the country's mandatory fortification program, but rather for the explicit purposes of conducting the research project. Two, the results were presented combined, for all foods together, and it was not possible to isolate the contribution of fortified wheat flour.

For example, Best and colleagues (2011) reviewed the impact of foods fortified with multiple micronutrients on many health outcomes including

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Table 14 Results from modeling the health impact of wheat flour fortification, alone or in combination with maize flour, for multiple countries

Study	Countries (n)	Nutrient[a]	Health outcome[b]	Individuals studied	Results
Youngblood et al. 2013	65	Folic acid	Neural tube defects	Live births	In 2012, flour fortification with folic acid prevented 25% of neural tube defects[c]
Arth et al. 2016	58	Folic acid	Neural tube defects	Live births	In 2015, flour fortification with folic acid prevented 13.2% of neural tube defects[c]
Kancherla et al. 2018	59	Folic acid	Neural tube defects	Live births	In 2017, flour fortification with folic acid prevented 18% of neural tube defects[c]

[a] Nutrient added to wheat flour through fortification which is purported to affect the health outcome.
[b] Some of the health outcomes measure the concentration of nutrients or other constituents in the blood or breastmilk and some refer to the prevalence (i.e. percentage of people who have the condition), the incidence (i.e. number of people newly diagnosed with the condition), or deaths (i.e. number of people who die due to the condition).
[c] These are the neural tube defects that can be prevented by women having optimum blood folate levels around the time of conception, known as folic acid preventable spina bifida and anencephaly.

Table 15 Systematic reviews or meta-analyses that evaluated wheat flour in the assessment of fortification's health impact and reason for exclusion from this chapter

Study	Nutrient[a]	Health outcome[b]	Reason for exclusion[c]
Athe et al. 2014	Iron	Hemoglobin	Effect of wheat flour could not be isolated
Best et al. 2011	Multiple	Multiple	Flour not fortified per mandatory fortification program
Black et al. 2012	Vitamin D	Vitamin D status	Flour not fortified per mandatory fortification program
Castillo-Lancellotti et al. 2012	Folic acid	Breast cancer risk	Effect of wheat flour could not be isolated
Chen et al. 2014	Folic acid	Breast cancer risk	Effect of wheat flour could not be isolated
Centeno Tablante et al. 2019	Folic acid	Multiple	For most studies, flour not fortified per mandatory fortification program
Eichler et al. 2019	Multiple	Multiple	For most studies, flour not fortified per mandatory fortification program
Gera et al. 2012	Iron	Multiple	Effect of wheat flour could not be isolated
Ghanchi et al. 2019	Iron	Diarrhea	Flour not fortified per mandatory fortification program
Hess et al. 2016	Multiple	Multiple	Flour not fortified per mandatory fortification program
Hombali et al. 2019	Vitamin A	Multiple	Effect of wheat flour could not be isolated
Kennedy et al. 2011	Folic acid	Colorectal cancer	Effect of wheat flour could not be isolated
Mendu et al. 2019	Vitamin A	Vitamin A status	Effect of wheat flour could not be isolated
O'Donnell et al. 2008	Vitamin D	Vitamin D status	Effect of wheat flour could not be isolated
Petry et al. 2016	Iron, Zinc	Multiple	Effect of wheat flour could not be isolated
Salam et al. 2019	Multiple	Multiple	Effect of wheat flour could not be isolated
Shah et al. 2016	Zinc	Multiple	Flour not fortified per mandatory fortification program
Tam et al. 2020	Multiple	Multiple	Effect of wheat flour could not be isolated

Study	Nutrient[a]	Health outcome[b]	Reason for exclusion[c]
Tio et al. 2014	Folic acid	Breast cancer risk	Effect of wheat flour could not be isolated
Yang et al. 2016	Folic acid	Multiple	Effect of wheat flour could not be isolated

[a] Nutrient added to foods through fortification which is purported to affect the health outcome.
[b] Some of the health outcomes measure the concentration of nutrients or other constituents in the blood or breastmilk and some refer to the prevalence (i.e. percentage of people who have the condition), the incidence (i.e. number of people newly diagnosed with the condition), or deaths (i.e. number of people who die due to the condition).
[c] Reason why the research was excluded from this chapter: (1) the wheat flour was not fortified under the rubric of the country's mandatory fortification program and/or (2) the results were presented combined for all fortified foods and the independent contribution of fortified wheat flour could not be isolated.

nutritional status, growth and cognitive development. Of 12 studies included in their review, two assessed the impact of fortified biscuits. Both of these studies used flour that was fortified to meet the researchers' scientific interests; the flour was not fortified according to the country's mandatory fortification program (or the country did not have such a national program at that time).

7 Summary

- The health impact of wheat flour fortification after large-scale implementation in countries has been studied employing different study designs and in a diversity of health outcomes that span all age groups and many systems of the human body.
- Folic acid was the most studied nutrient. To a lesser extent, fortification with iron, vitamin B12 and zinc was also examined.
- The most studied outcomes were neural tube defects, cancer, folate status, folate deficiency, anemia, iron deficiency, iron status, hemoglobin and iron-deficiency anemia.
- For all of these outcomes except iron-deficiency anemia, the majority of studies showed improvements after fortification. That is, these studies suggest that fortification reduces neural tube defects, cancer, folate deficiency, anemia, and iron deficiency, and that fortification improves folate status, iron status, and hemoglobin levels.
- For some of the outcomes (cancer, anemia, hemoglobin, folate deficiency), there were studies that indicated health outcomes worsened after fortification. These analyses suggest that cancer, anemia and folate deficiency increased and hemoglobin levels decreased after fortification.
- Discrepant values for cancer may be due to the difference in years since fortification was initiated (e.g. increased incidence of colon cancer after fortification is suggested by studies published in the 2000s; the opposite

is observed in studies published in the 2010s) or by the sample size in studies (e.g. increased breast cancer incidence after fortification is observed in studies with sample sizes <2000; studies that observed no difference or a decreased incidence after fortification have sample sizes >2000 and going into the millions).

- Discrepant values for anemia and hemoglobin may be due to the (1) existence of non-nutritional causes of anemia which cannot be addressed by fortification, (2) nutritional causes of anemia not addressed by fortification because a limited number of nutrients were added through fortification and (3) levels of nutrients or fortification compounds used in fortification do not follow international guidelines.
- Many more outcomes were studied which only have results for four or fewer analyses: folate-deficiency anemia, homocysteine status, high homocysteine, vitamin B12 deficiency masking, congenital heart disease, coronary heart disease, stroke, myocardial infarction, cognitive function, hypersensitivity, thyroid- and diabetes-related disorders, orofacial clefts, vitamin B12 status, vitamin B12 deficiency, zinc status and zinc deficiency.
- Homocysteine status, high homocysteine, stroke death, myocardial infarction, orofacial clefts, vitamin B12 status, vitamin B12 deficiency, zinc status and zinc deficiency trend toward improvement after fortification.
- Vitamin B12 deficiency masking and cognitive function trend toward showing no difference before and after fortification.
- None of the outcomes with two, three or four analyses shows a worsening of health after fortification.
- While none of the study designs employed can be used to confirm a causal relationship between fortification and health outcomes, the preponderance of the evidence suggests that wheat flour fortification improves many health outcomes.
- Because fortification may also be associated with negative health outcomes such as cancer, health monitoring should continue to assess these outcomes in countries with fortification.

8 Future trends in research

Program decision makers are urged to consider several actions that can facilitate the health impact evaluation of their flour fortification programs:

- Plan for health impact evaluations while planning for the implementation of fortification.
- Use existing data or data-collection systems to evaluate the health impact of fortification at lower cost than planning a stand-alone fortification evaluation.

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- Regularly review all kinds of information on the fortification program to determine if it is likely to be having health impact, such as intake, coverage and compliance. And, review this information before proceeding with an impact evaluation.
- Craft program impact pathways to document the plausibility that fortification contributed to the health outcome observed.
- Make fortification information publicly available for scrutiny by interested parties.
- Ensure that multiple programs (such as fortification, supplementation, micronutrient powders, biofortification) are not providing an excess of nutrients to the population. If they are, review and adjust programs accordingly.
- Continue to assess for potential negative health outcomes of fortification. This is especially important for outcomes that may take years or decades to manifest, such as cancer.

9 Where to look for further information

9.1 World Health Organization guidelines

The World Health Organization (WHO) offers several guidelines related to food fortification generally and wheat flour fortification specifically.

WHO's website on wheat flour fortification.

WHO. e-Library of Evidence for Nutrition Actions (eLENA): fortification of wheat flour. https://www.who.int/elena/titles/wheat-flour-fortification/en/.

WHO and FAO's book with basic principles of food fortification.

WHO and FAO. (2006). Guidelines on food fortification with micronutrients. https://www.who.int/nutrition/publications/micronutrients/9241594012/en/.

WHO's recommendations for wheat and maize flour fortification.

World Health Organization, Food and Agriculture Organization of the United Nations, the United Nations Children's Fund, Global Alliance for Food Fortification, Micronutrient Initiative, and the Food Fortification Initiative. (2009). Recommendations on wheat and maize flour fortification. Meeting report: interim consensus statement. https://www.who.int/nutrition/publications/micronutrients/wheat_maize_fortification/en/.

Special issue of the Food and Nutrition Bulletin journal that summarizes the evidence that generated WHO's 2009 recommendations.

(2010). The opportunity of flour fortification: building on the evidence to move forward. https://journals.sagepub.com/toc/fnba/31/1_suppl1.

9.2 Flour Millers toolkit

The Food Fortification Initiative provides basic specifications for fortifying flour at the wheat mill.

Food Fortification Initiative. Flour Millers Toolkit for implementing wheat and maize flour fortification. http://www.ffinetwork.org/tools_training/flour_millers_toolkit.html.

9.3 Best practices for foundational fortification documents

A review of best practices for fortification legislation and standard documents and monitoring protocols and an assessment of how closely countries with mandatory fortification of wheat flour, maize flour and rice follow these best practices.

Marks, K. J. et al. (2018), Review of grain fortification legislation, standards, and monitoring documents. https://www.ghspjournal.org/content/6/2/356.

9.4 Government monitoring of fortification

Monitoring by governments is essential to ensure flour is adequately and consistently fortified. This document provides guidance on the minimum elements that should be included in a country's monitoring plan.

Global Alliance for Improved Nutrition and Project Healthy Children. (2018). Regulatory monitoring of national food fortification programs: A policy guidance document. https://fortificationdata.org/resources/.

9.5 Country statistics on wheat flour fortification

These can be found at two websites:

Global Fortification Data Exchange. https://fortificationdata.org.
Food Fortification Initiative. http://www.ffinetwork.org.

9.6 Book on food fortification

A recently published book on fortification offers over 40 chapters on different aspects of food fortification, including health impact evaluations.

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Mannar, M. G. V. and Hurrell, R. F. (2018), Food fortification in a globalized world. https://www.elsevier.com/books/food-fortification-in-a-globalized-world/mannar/978-0-12-802861-2.

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