

Systematic Review

Not all cases of neural-tube defect can be prevented by increasing the intake of folic acid

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Some countries have introduced mandatory folic acid fortification, whereas others support periconceptional supplementation of women in childbearing age. Several European countries are considering whether to adopt a fortification policy. Projections of the possible beneficial effects of increased folic acid intake assume that the measure will result in a considerable reduction in neural-tube defects (NTD) in the target population. Therefore, the objective of the present study is to evaluate the beneficial effects of different levels of folic acid administration on the prevalence of NTD. Countries with mandatory fortification achieved a significant increase in folate intake and a significant decline in the prevalence of NTD. This was also true for supplementation trials. However, the prevalence of NTD at birth declined to approximately five cases at birth per 10 000 births and seven to eight cases at birth or abortion per 10 000 births. This decline was independent of the amount of folic acid administered and apparently reveals a 'floor effect' for folic acid-preventable NTD. This clearly shows that not all cases of NTD are preventable by increasing the folate intake. The relative decline depends on the initial NTD rate. Countries with NTD prevalence close to the observed floor may have much smaller reductions in NTD rates with folic acid fortification. Additionally, potential adverse effects of fortification on other vulnerable population groups have to be seriously considered. Policy decisions concerning national mandatory fortification programmes must take into account realistically projected benefits as well as the evidence of risks to all vulnerable groups.

Folic acid: Fortification: Supplementation: Neural-tube defect

Neural-tube defects (NTD) are common congenital malformations that can lead to severe disability or even death. The causes of NTD are multifactorial, including genetic predisposition, environmental risk factors and maternal conditions⁽¹⁾. The risk of spina bifida and anencephaly can be reduced significantly by increasing the folate intake before and during the first 28 d after conception^(2,3). Around the world, this was the rationale for the implementation of public-health programmes to increase the folate status of women. These approaches include national public education campaigns, recommendation of folic acid supplementation for all women of childbearing age as well as a voluntary or mandatory folic acid fortification of food⁽⁴⁾. The USA, Canada (since 1998) and Chile (since 2000) have a nutrition policy of mandatory fortification of enriched cereal-grain products, whereas, in Europe, no country has yet required a mandatory folic acid fortification. Hungary has introduced a non-compulsory bread fortification programme with folic

acid, vitamin B₁₂ and vitamin B₆⁽⁵⁾. Australia and New Zealand also introduced a voluntary fortification programme of some foods with folic acid in 1995 and mandatory fortification of a staple food is in the process of approval⁽⁶⁾. Fortification policies differ widely in Europe. Some countries (e.g. Germany) allow fortification of flour and breakfast cereals, whereas other countries (e.g. The Netherlands) have a strict prohibition of folic acid addition to foods⁽⁷⁾. At present, national scientific committees in several European countries (e.g. Germany, Ireland, The Netherlands, UK) have recommended mandatory folic acid fortification of flour or bread, but the implementation process has not yet been established^(8–11). A national fortification programme is a public-health initiative undertaken only in the light of significant scientific findings relating a specific nutrient deficiency to a specific disease condition⁽¹²⁾.

Worldwide, many countries support the periconceptional folic acid supplementation of women who might become pregnant as a

Abbreviation: NTD, neural-tube defect.

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well-defined target group. These educational health campaigns have been shown to be useful, but until now have been only partially effective⁽⁴⁾ with often limited impact⁽¹³⁾. The widespread low health literacy of people, ineffective communication strategies and inadequate support by physicians and other relevant health professionals may have attributed to this. The limited efficacy of the campaigns to promote supplementation among women who might become pregnant is what continues to drive discussions that call for widespread mandatory fortification for all segments of the population.

Consequently, there is much discussion and emotional pressure in many European countries to implement mandatory folic acid fortification because NTD are such severe congenital birth defects and infants with spina bifida are likely to have lifelong disabilities. Despite the observed beneficial effects of folic acid fortification on the folate status and the prevalence of NTD, there are some suspected adverse effects⁽¹⁴⁾. Moreover, the proven protection against birth defects that is conveyed by fortification has also been hypothesised to be accompanied by protective effects against CVD and cancer in both men and women, and this may have influenced fortification policies.

In extrapolations of the expected benefits of folic acid fortification, it is sometimes directly and more often indirectly assumed that nearly all causes of NTD are preventable by this measure. However, a biological rationale for this assumption is lacking. Genetic variants and disorders as well as environmental risk factors, such as periconceptional clomiphene use, other drugs, alcohol, assisted reproductive technology, periconceptional vitamin B₁₂ deficiency and maternal obesity, are also associated with NTD^(15,16).

The overall experiences among countries with a fortification policy or specific recommendations of using folic acid supplements during pregnancy, in conjunction with the data from randomised controlled trials as well as from observational studies, can be used to re-evaluate the true benefits of an increased intake of folic acid on NTD risk reduction. Therefore, the aim of the present study is to analyse the potential impact of different means to increase the intake of folic acid on the incidence of spina bifida and anencephaly.

Subjects and methods

Literature search

In a MEDLINE (Database from the National Library of Medicine, Bethesda, USA) search, papers were identified which reported the effects of increasing the folate intake on NTD. The search was extended to papers describing the potential implications of mandatory folic acid fortification. The keywords included are 'folic acid', 'folate' and 'NTD' in alternating combinations with 'fortification' 'intervention study', 'trial' and 'RCT' (randomised controlled trial). We also checked the reference lists for additional relevant studies. Supplemental data were collected from the regional and national birth-defect registries. Case-control studies were not considered because no prevalence data could be calculated from these studies. Moreover, case-control studies may overestimate the protective or adverse effect of folic acid⁽¹⁷⁾.

When evaluating the effect of increased folic acid on the outcome NTD (the aggregation of spina bifida, anencephaly

and encephalocele), we grouped the studies based on three different types of outcomes:

- (i) studies measuring the prevalence of NTD in recurrence trials in women with a previous NTD-affected pregnancy,
- (ii) studies measuring the prevalence of NTD only at birth,
- (iii) studies measuring the prevalence of NTD from spontaneous or induced abortions and at birth.

Results

Neural-tube defects

It is well established that women who have had a pregnancy affected by an NTD have a significant elevated risk of a subsequent NTD-affected pregnancy⁽¹⁸⁾. The average risk of recurrence in NTD was calculated to be 4%⁽¹⁹⁾. The methylenetetrahydrofolate reductase C677T single-nucleotide polymorphism, as well as some other less common genotypes, are genetic risk factors for NTD in both infants with NTD and their mothers⁽¹⁾. It has been speculated that mothers with genetic polymorphisms may have a need for a much higher preventive intake of folate. Therefore, the data derived from study groups with a deviant genetic composition (e.g. recurrence trials) are not applicable to the general population. Also, measuring the prevalence of NTD in a population by diagnosing the cases from spontaneous or induced abortions and at birth, of course, results in much higher estimates of prevalence than deriving them from NTD cases at birth only⁽¹⁷⁾. Most available data on NTD prevalence are from passive surveillance systems and only few from active systems. Active surveillance systems report approximately twice as many cases of major birth defects than passive systems⁽²⁰⁾. For methodological reasons, only comparable data from passive surveillance systems are included in the present study.

Recurrence trials. In the classic Medical Research Council study, a randomised double-blind recurrence trial, conducted in seven countries worldwide, the periconceptional application of 4 mg folic acid to women having a previous NTD-affected pregnancy resulted in a significant difference ($P < 0.05$) between the folic acid group (101 cases per 10 000) and the control group (349 cases per 10 000), meaning a 72% protective effect. NTD was diagnosed in fetus or infant⁽²⁾.

Another trial was carried out at five centres in India. Periconceptional application of a multivitamin preparation containing 4 mg folic acid was evaluated for its efficacy in preventing the recurrence of NTD in a blind, placebo-controlled randomised trial⁽²¹⁾. Cases of NTD were diagnosed by antenatal screening or at birth. The recurrence of NTD in the vitamin group was 292 cases per 10 000 births when compared with 704 cases per 10 000 births in the placebo group. Overall, a difference of 58% was observed between the supplemental and control groups ($P = 0.06$).

In both recurrence trials, the prevalence in the untreated group was very high, reflecting the elevated risk in women with a previous NTD-affected pregnancy. Compared with the MRC study, the prevalence of NTD in the Indian study before and after the intervention was much higher, although the same supplemental dose of folic acid was used (Fig. 1). The differences in compliance, genetics and other folic

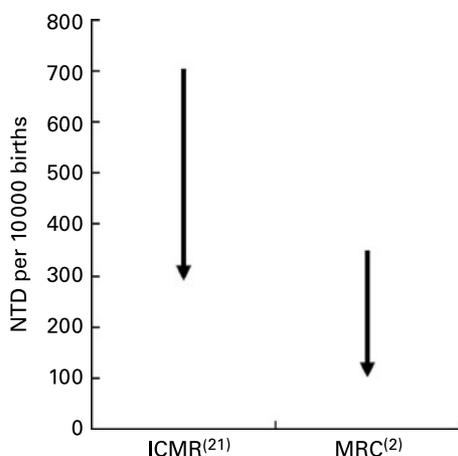


Fig. 1. Decline in neural-tube defects (NTD) by the periconceptional application of folic acid (4 mg/d) in randomised controlled recurrence trials.

acid-independent reasons can be assumed. Additionally, the traditional low intake of vitamin B₁₂ in India has to be considered.

It is well established that women with an elevated NTD risk can reduce their risk in subsequent pregnancy considerably by taking a high dose of 4 mg folic acid/d around the time of conception.

In a recent meta-analysis of randomised trials of folic acid for the prevention of recurrent NTD, it was calculated that a 69% reduction in recurrence risk was achieved if analysed on an intention-to-treat basis and a 87% reduction among those women who took supplements before the beginning of pregnancy⁽¹⁹⁾. However, the efficacy of lower dosages (e.g. <1–4 mg/d) has never been tested in intervention studies of recurrent NTD. In addition to a genetic basis, it has been hypothesised that this high dose of folic acid is required to overcome the effect of antibodies against folate receptors, which were detected in the serum of women with a pregnancy complicated by a NTD⁽²²⁾.

Intervention studies with neural-tube defect measured at birth. The implementation of mandatory folic acid fortification in Canada, Chile and the USA provides the opportunity to study the effect of fortification in population groups with very

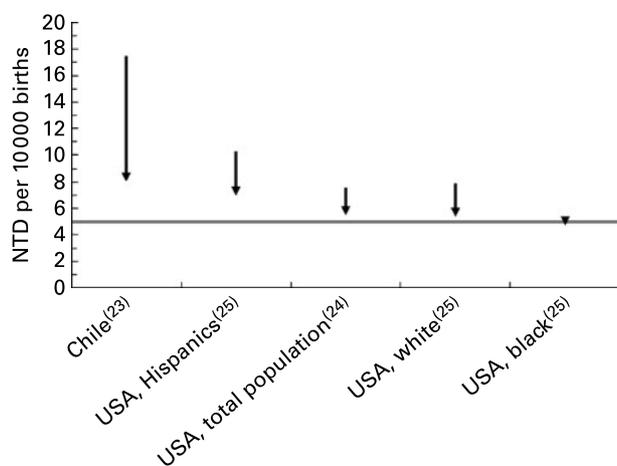


Fig. 2. Decline in neural-tube defects (NTD) prevalence after mandatory folic acid fortification (NTD diagnosed only at birth).

different prevalence rates of pre-interventional NTD. Only studies reporting prevalence rates directly before or after the intervention are included (Fig. 2).

Chile started to fortify wheat flour in a mandatory fashion with folic acid in 2000, adding 2.2 mg/kg and providing approximately 400 µg additional folic acid per capita. Consequently, a decline in NTD from 17.5 to 8.0 cases per 10 000 births between the pre- (1992–2000) and post-fortification periods (2001–2002) was observed⁽²³⁾. Overall, the baseline prevalence of NTD births was rather high in Chile compared with countries with therapeutic abortions, because the prevalence was not influenced by incomplete ascertainment due to terminations of pregnancy (induced abortion is illegal in Chile). No secular trend was seen in the pre-fortification period.

In the USA, mandatory folic acid fortification of enriched grain products also resulted in significant differences in the prevalence of NTD in the general population and among specific racial/ethnic groups. Comparing the NTD rates in the pre-fortification period (1995–1996) with the post-fortification period (1999–2000), a decline in NTD-affected live births and stillbirths from 7.6 to 5.5 NTD per 10 000 births occurred⁽²⁴⁾. Nevertheless, the observed difference (–26%) was important but was less than the originally projected decline of 50%. An examination of the specific racial/ethnic groups provides more detailed insights into fortification's impact on population groups with a different NTD burden. The highest baseline prevalence of spina bifida and anencephaly was seen in the USA among Hispanic births, followed by non-Hispanic white births, while the lowest prevalence is observed among non-Hispanic black births. Among Hispanic women, mandatory fortification was associated with a decline in NTD-affected births from 10.3 to 7.0 NTD per 10 000 births (–32%). Among non-Hispanic white women, a decline from 7.9 to 5.4 NTD per 10 000 births (–32%) was observed, whereas in non-Hispanic black women, only a decline from 5.4 to 4.7 NTD per 10 000 births (–13%) occurred⁽²⁵⁾. Thus, even within the confines of the USA population, a smaller effect of fortification was realised among the racial groups that had lower rates of NTD births at baseline.

Intervention studies with neural-tube defect measured at birth or abortion. Measuring NTD only at birth underestimates the severity of the problem. Therefore, studies measuring NTD in fetuses from spontaneous or terminated abortions and at birth provide a more complete picture. In the identified intervention studies of NTD diagnosed at birth or abortion, the folic acid intake from supplements or fortification varied between 100 µg/d and 4 mg/d (Fig. 3).

As part of a public-health campaign conducted between 1993 and 1995 in two Chinese regions with higher (northern) and lower (southern) NTD rates, the impact of periconceptional folic acid supplementation (+400 µg/d) on the outcomes of pregnancy was evaluated in a non-randomised intervention study. Northern China is a region with a traditional lower intake of folate-rich vegetables. In northern China, the intervention resulted in a remarkable decline from forty-eight to seven cases of NTD per 10 000 births (–80%). At the same time, in southern China, the same amount of folic acid resulted in a decline from ten to six NTD per 10 000 births (41%)⁽³⁾.

In Canada, the eastern provinces had a higher baseline NTD prevalence than the western provinces. Assessing the changes

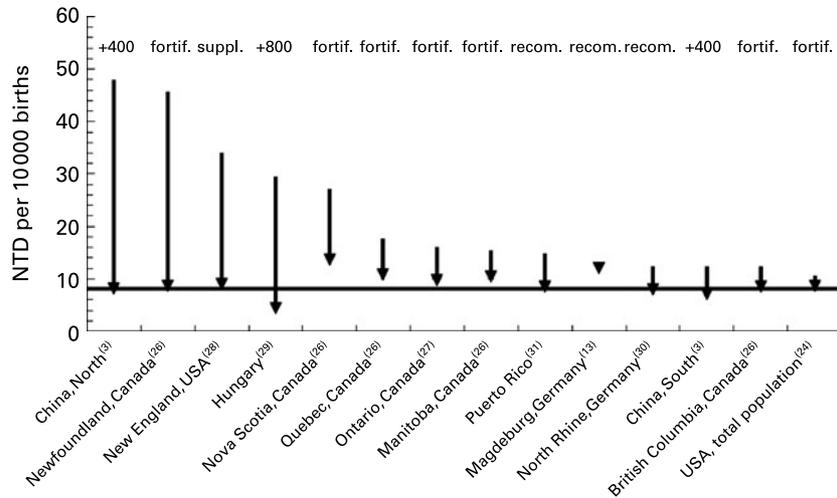


Fig. 3. Decline in neural-tube defects (NTD) by the periconceptional application of folic acid in primary intervention trials or after fortification (NTD diagnosed at birth or abortion). + 400 (+800), μg folic acid/d in randomised controlled trials, verum v. placebo group; fortif., before and after the mandatory fortification of cereal-grain products; suppl., cohort studies, multivitamin (including folic acid) users v. non-users; recom., before and after the recommendation of periconceptional use of folic acid by professional bodies.

in NTD before and after the implementation of mandatory folic acid fortification with an estimated additional daily intake of $150 \mu\text{g}$ folic acid shows significant reductions in all provinces. NTD prevalence declined in Newfoundland and Labrador from 45.6 to 7.6 cases per 10 000 births (-83%), in Nova Scotia from 27.2 to 12.6 cases per 10 000 births (-54%), in Quebec from 17.7 to 9.7 cases per 10 000 births (-45%), in Manitoba from 15.4 to 9.3 cases per 10 000 births (-40%), in Alberta from 11.2 to 6.7 cases per 10 000 births (40%) and in British Columbia from 9.6 to 7.5 per 10 000 births (-22%)⁽²⁶⁾. Thus, as was true of intra-societal comparisons in the USA and China, the decline in Canada was much more pronounced in regions with a higher baseline prevalence of NTD than in regions with a lower baseline prevalence. A linear relationship between the baseline prevalence of NTD and the magnitude of the decrease after the fortification was implemented was observed. A separate study conducted in Ontario showed a decline from 16.2 to 8.6 cases per 10 000 births (-47%)⁽²⁷⁾.

A re-evaluation of an observational study of early prenatal exposures with folic acid containing multivitamin supplements and pregnancy outcomes, conducted from 1984 to 1987 in the northeastern USA, showed a prevalence of thirty-four cases per 10 000 births in the subgroup with a daily total folate intake of $< 150 \mu\text{g}$, compared with only eight cases per 10 000 births in the subgroup with a total intake of $> 1200 \mu\text{g}$ ⁽²⁸⁾.

In a Hungarian cohort-controlled trial conducted from 1993 to 1996, the periconceptional supplementation with $800 \mu\text{g}$ folic acid resulted in a prevalence of 3.3 cases per 10 000 births in the supplemented cohort and 29.4 cases per 10 000 births in the unsupplemented cohort, showing a difference of 89% ⁽²⁹⁾.

Before the recommendation of daily folic acid supplements ($+400 \mu\text{g}$) to all women in childbearing age in the German state Saxony-Anhalt, an NTD prevalence of 12.8 cases per 10 000 births was observed, while after the official recommendation, 10.9 cases per 10 000 births were detected⁽¹³⁾. A similar study in the German region North Rhine revealed 10.5 cases per 10 000 births in the prior and 6.8 cases per 10 000

births in the post-recommendation period⁽³⁰⁾. In Puerto Rico, the prevalence of NTD declined significantly from 14.7 cases per 10 000 births in 1996 to 7.5 cases per 10 000 births in the post-recommendation period⁽³¹⁾.

In the USA, the effect of folic acid fortification was studied in eight population-based surveillance systems, diagnosing NTD cases at birth or abortion. Comparing the NTD rates in the pre-fortification period (1995–1996) with the post-fortification period (1999–2000), a decline in NTD-affected live births and stillbirths from 10.6 to 7.6 NTD per 10 000 births (-28%) occurred⁽²⁵⁾.

Prevalence of neural-tube defect without intervention. Prevalence of NTD is available from the national or at least regional birth-defect registries in Europe, especially from the European Registration of Congenital Anomalies and Twins, a network of population-based registries in Europe⁽³²⁾. NTD cases were ascertained among live-born infants, stillbirths and pregnancy terminations. Fig. 4 shows that the prevalence of NTD in seven countries is below eight cases per 10 000 births.

The data from Mainz (Germany) were not included in Fig. 4, because this registry used an active surveillance system for registering malformations, which is totally different from the system of other European Registration of Congenital Anomalies and Twins registries⁽³³⁾. Thus, many countries in Europe generally have low basal prevalence of NTD births and would therefore be expected to realise a very modest benefit from the fortification if the afore-mentioned relationship held true.

Folate intake and status after fortification. The mandatory folic acid fortification in the USA was predicted to increase the mean folate intake by approximately $100 \mu\text{g}/\text{d}$ ⁽³⁴⁾. Studies comparing the effect of fortification on the folate intake and status differ from these estimations significantly, suggesting as much as $200 \mu\text{g}/\text{d}$ folic acid from fortified food in the USA⁽³⁵⁾ with large race/ethnicity differences. At the same time, the prevalence of folate intake above the safe upper limit of $1000 \mu\text{g}/\text{d}$ increases from 1.3 to 11.3%⁽³⁶⁾. Soon after the implementation of folic acid fortification (1999–

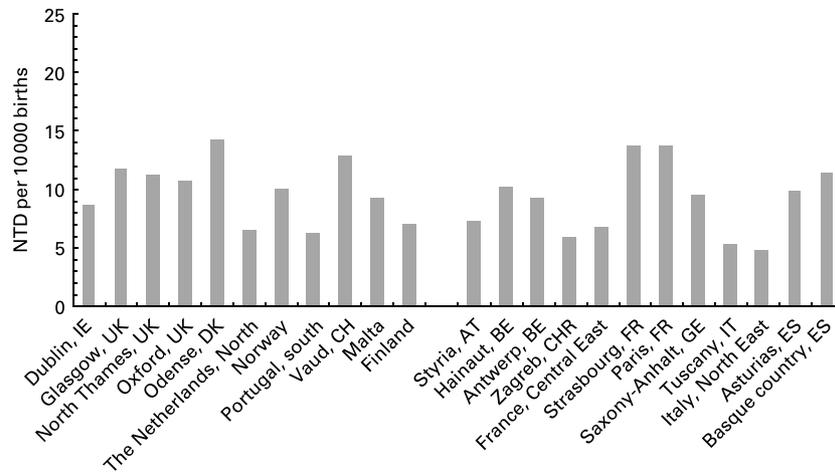


Fig. 4. Neural-tube defects prevalence (at birth or abortion) in Europe from birth-defect registries from 2000 to 2002⁽³²⁾. IE, Republic of Ireland; DK, Denmark; CH, Switzerland; AT, Austria; BE, Belgium; CHR, Croatia; FR, France; GE, Germany; IT, Italy; ES, Spain.

2000), the median folate concentration in the serum has more than doubled (+156%) and in erythrocytes increased by 59%^(37,38).

Changes in eating habits and a reduction in the degree of folic acid 'overage' in enriched breads probably resulted in a slight decrease in folate status since the initial implementation of fortification in the USA⁽³⁹⁾. In addition, it cannot be excluded completely that intermittent modifications in folate analysis have occurred and influenced the observed changes. However, in the period 2003–2004, median folate concentrations in the plasma were still 116% and in erythrocyte 46% above baseline. The prevalence estimates of low serum and erythrocyte folate concentrations in women of childbearing age from pre- to post-fortification declined from 21 to <1% and from 38 to 5%, respectively⁽⁴⁰⁾.

Discussion

Without any doubt, folic acid fortification of grain products results in a significant increase in folate intake and status in the general population, as well as a reduction in NTD. However, the array of studies consistently shows that not all cases of NTD are preventable by increasing the folate intake. As shown in Figs. 2 and 3, a greater reduction occurs in the population groups with a higher baseline prevalence, whereas in the study groups with a comparatively low baseline rate, a much smaller reduction is achievable. Independent of the baseline rates, all the study groups had a residual prevalence of approximately five cases of NTD per 10 000 births after fortification when NTD was measured at birth only. When measuring the NTD prevalence at birth or from abortion after folic acid application in controlled trials, approximately seven to eight cases of NTD per 10 000 births are still observed after the implementation of fortification or after the periconceptional use of supplements. These observations collectively demonstrate in a rather clear fashion that the degree of reduction in NTD prevalence in a population affected by folate is related to the baseline NTD prevalence and further indicate a low level of NTD births below which folate does not appear to be effective, regardless of the dose.

An analysis of the USA data by race/ethnicity clearly shows that a significant decline was only observed among ethnic groups with an NTD prevalence above the average in the pre-fortification period, that is in non-Hispanic white (–32%) and Hispanic (–32%) but not in non-Hispanic black (–13%)⁽²⁴⁾. After mandatory fortification, the prevalence of NTD shows a smaller variation between the racial/ethnic groups (Hispanics, 7.0/10 000; non-Hispanic white, 5.4/10 000; non-Hispanic black, 4.7/10 000). The remaining disparity may reflect genetically determined differences. One possibility is the C677T polymorphism in the methylenetetrahydrofolate reductase gene, which occurs more frequently among Hispanics compared with white people, who have an intermediate frequency, and black people with the lowest frequency of polymorphism⁽⁴¹⁾. Additionally, polymorphisms of other folate-dependent enzymes and other genetic variants⁽¹⁵⁾, as well as environmental factors, might be responsible for the differences in NTD risk still observed among the racial/ethnic groups after fortification⁽²⁴⁾.

While the relative decline in NTD prevalence is dependent on the baseline, setting a target of reducing the NTD prevalence by 50% – an objective in the US framework 'Health People 2010' – is highly misleading. The Centers for Disease Control and prevention report that the prevalence has fallen only by 26% in the USA after food fortification with folic acid⁽²⁵⁾ is probably attributable to the relative low NTD prevalence of the general population in the pre-fortification period, and not to the level of folic acid in fortified foods as suggested by some⁽⁴²⁾. Also, the recently published notion that up to 70% of NTD can be prevented by folic acid fortification⁽³⁵⁾ inappropriately have employed a percentage target reduction rather than a goal of the lowest rate of NTD births that can be achieved by folic acid fortification. Moreover, the demand of some authors⁽⁴³⁾ to increase the fortification level in the USA seems to be therefore unjustified against the background of the presented data⁽⁴⁴⁾.

The potential benefit in the NTD prevention among the countries without folic acid fortification programmes that have an NTD prevalence close to the observed floor is likely to be considerably smaller than what is usually predicted. Additionally, in many countries, a pre-existing decline

in the prevalence of NTD has been observed before any intervention to increase the folate status was implemented^(13,45).

Although the explanation that folate-independent risk factors are responsible for the apparent floor effect is very likely, other possible interpretations have to be taken into account. One interpretation could be that, in all interventions, some participants were not or at least not fully compliant with the study protocol. However, the observation that different measures to increase the folate intake result in consistent post-intervention NTD prevalences (Fig. 3) would appear to contradict this interpretation. Moreover, the mandatory fortification of flour gives little opportunity for non-compliance, but still supports the concept of a floor effect.

Besides these findings, meta-analyses have shown no conclusive evidence of benefit for folic acid supplementation commenced after the first trimester of pregnancy. Therefore, no significant effects can be expected when periconceptual folic acid supplements are continued through pregnancy⁽⁴⁶⁾. Although it is often hypothesised, folic acid supplementation failed to reduce substantially the risk for other major malformations other than NTD^(13,47). A recent systematic review and meta-analysis also found no strong evidence that folate alone is associated with oral cleft aetiology⁽⁴⁸⁾.

The biological mechanism of NTD development, how folate influences this mechanism and the precise folic acid dose required to exert its protective effect are still uncertain. Lawrence & Riddell⁽⁴⁹⁾ pointed out that the protective effect seems to be more consistent with a therapeutic-type response rather than addressing a conventional folate deficiency.

An overall evaluation of the benefits and risks of a mandatory fortification of food with folic acid has to review seriously the existing body of evidence and derived hypotheses on potential adverse effects of other vulnerable population groups^(50,51). For example, the hypothesised role of folic acid on cancer progression and recurrence, as well as the overall dual role of folate in carcinogenesis, could have implications in the ongoing debate in Europe concerning mandatory folic acid fortification of foods^(52,53). Meanwhile, another potential food fortification (5-methyltetrahydrofolic acid) is available, which might avoid the potential risks of folic acid.

Besides, a high intake of folic acid has been associated with a faster rate of cognitive decline and anaemia in elderly people with a poor vitamin B₁₂ status. By contrast, among the subjects with a normal vitamin B₁₂ status, high serum folate concentrations were associated with a protection from cognitive impairment⁽⁵⁴⁾. These findings have recently been reinforced from a metabolic perspective by the demonstration that among people with low serum vitamin B₁₂ concentrations, high plasma folate levels were associated with higher concentrations of homocysteine and methylmalonic acid, two functional indicators of impaired vitamin B₁₂ status⁽⁵⁵⁾.

Moreover, a meta-analysis of randomised controlled trials has failed to demonstrate a benefit of folic acid supplementation to reduce the risk of CVD or all-cause mortality among participants with prior history of vascular disease by lowering the homocysteine levels⁽⁵⁶⁾.

Conclusions

Supplementation with folic acid in the appropriate framework reduces the risk of NTD in a very convincing fashion. Apart

from this beneficial effect of folic acid fortification on NTD risk, there are two important issues that one needs to bear in mind. First, comparisons of NTD reduction between ethnic groups or countries indicate that not all cases of NTD are preventable by increasing the folate intake. The countries with an NTD prevalence of four to five cases at birth per 10 000 births or seven to eight cases at birth or abortion per 10 000 births might have already reached the floor of folic acid-preventable NTD. Second, mandatory fortification results in a higher intake of folic acid for the whole population and exposing large non-target groups to high levels of folic acid might have unintended beneficial or harmful effects in large numbers of individuals.

It is particularly important that in an issue as complex and challenging as this one, the ethical and potential risk implications have to be considered carefully. Mandatory folic acid fortification of food is facing scientific uncertainties. On the one hand, uncertainties in the evaluation of potential risks and benefits arise because presumed genetic abnormalities in at-risk individuals are addressed with a population-wide intervention. On the other hand, the existing body of evidence and derived hypotheses on potential adverse effects of other vulnerable population groups affected by mandatory folic acid fortification have to be taken seriously.

Impending policy decisions concerning fortification programmes may be delayed, as realistic projections of benefit and recent observations regarding adverse effects of fortification are evaluated. In the meantime, women who might become pregnant should be encouraged by their physicians and by greater public education to use the periconceptual folic acid supplementation. Policy makers should be convinced to support the distribution of folic acid and vitamin B₁₂ supplements to this well-defined target group⁽⁵⁷⁾. Therefore, authorities should invest more resources supporting programmes for targeted folic acid supplementation and more efficient public education on the importance of periconceptual use of folic acid, as well as prompt a thorough aetiological investigation and genetic counselling.

Further research is needed to define the folate requirement of different groups with polymorphisms of genes involved in the folate metabolism, as well as of different geographical or ethnic groups. In the USA, mandatory folic acid fortification resulted in ethnic differences of NTD prevalences. It has to be clarified whether there are different 'floors' of NTD prevalence levels in different communities or whether these groups have different folate requirements. This might have implications for public-health policies in respect of periconceptual folic acid intake, and call for a more individual supplementation practice and less for a general fortification of a basic food source.

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References

- van der Linden IJ, Afman LA, Heil SG, *et al.* (2006) Genetic variation in genes of folate metabolism and neural-tube defect risk. *Proc Nutr Soc* **65**, 204–215.
- MRC Vitamin Study Research Group (1991) Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. *Lancet* **338**, 131–137.
- Berry RJ, Li Z, Erickson JD, *et al.* (1999) Prevention of neural-tube defects with folic acid in China. *N Engl J Med* **341**, 1485–1490.
- Buttriss J (2005) Strategies designed to increase awareness about folates and health and to increase folate intake: a review. *Trends Food Sci Technol* **16**, 246–252.
- Czeisel AE & Kökény M (2002) Bread is fortified with folic acid in Hungary. *Br Med J* **325**, 391.
- Bower C, Miller M, Payne J, *et al.* (2005) Promotion of folate for the prevention of neural tube defects: who benefits? *Paediatr Perinat Epidemiol* **19**, 435–444.
- Finglas PM, de Meer K, Molloy A, *et al.* (2006) Research goals for folate and related B vitamin in Europe. *Eur J Clin Nutr* **60**, 287–294.
- Food Safety Authority of Ireland (2006) *Report of the National Committee on Folic Acid Food Fortification*. Dublin: FSAI.
- Krawinkel M, Brönstrup A, Bechthold A, *et al.* (2006) Strategien zur Verbesserung der Folatver-sorgung in Deutschland – Nutzen und Risiken. *Ernaehrungs-Umschau* **53**, 468–479.
- SACN (2006) *Folate and Disease Prevention*. London: The Stationery Office.
- Food Standards Agency (2008) Folic Acid Fortification. <http://www.food.gov.uk/healthierating/folicfortification>
- White Junod S (2001) Folic Acid Fortification: Fact and Folly. *Food and Law Institute: update issues* 4, 5, 6. <http://www.fda.gov/oc/history/makinghistory/folicacid.html>
- Botto LD, Lisi A, Bower C, *et al.* (2006) Trends of selected malformations in relation to folic acid recommendations and fortification: an international assessment. *Birth Defects Res A Clin Mol Teratol* **76**, 693–705.
- Cornel MC, de Smit DJ & de Jong-van den Berg LTW (2005) Folic acid – the scientific base for a public health policy. *Reprod Toxicol* **20**, 411–415.
- Chen CP (2008) Syndromes, disorders and maternal risk factors associated with neural tube defects (IV). *Taiwan J Obstet Gynecol* **47**, 141–150.
- Mitchell LE (2005) Epidemiology of neural tube defects. *Am J Med Genet C Semin Med Genet* **135C**, 88–94.
- Mills JL & Signore C (2004) Neural tube defect rates before and after food fortification with folic acid. *Birth Defects Res* **70**, 844–845.
- Joò JG, Beke A, Papp Z, *et al.* (2007) Risk of recurrence in major central nervous system malformations between 1976 and 2005. *Prenat Diagn* **27**, 1028–1032.
- Grosse SD & Collins JS (2007) Folic acid supplementation and neural tube defect recurrence prevention. *Birth Defects Res A Clin Mol Teratol* **79**, 737–742.
- Queisser-Luft A, Stolz G, Wiesel A, *et al.* (2002) Malformations in newborn: results based on 30 940 infants and fetuses from Mainz congenital birth defect monitoring system (1990–1998). *Arch Gynecol Obstet* **266**, 163–167.
- ICMR, Central Technical Co-ordinating Unit (2000) Multicentric study of efficacy of periconceptional folic acid containing vitamin supplementation in prevention of open neural tube defects from India. *Indian J Med Res* **112**, 206–211.
- Rothenberg SP, da Costa MP, Sequeira JM, *et al.* (2004) Autoantibodies against folate receptors in women with a pregnancy complicated by a neural-tube defect. *N Engl J Med* **350**, 134–142.
- López-Camelo JS, Orioli IM, da Graça Dutra M, *et al.* (2005) Reduction of birth prevalence rates of neural tube defects after folic acid fortification in Chile. *Am J Med Genet* **135A**, 120–125.
- CDC (2004) Spina bifida and anencephaly before and after folic acid mandate – United States, 1995–1996 and 1999–2000. *MMWR Morb Mortal Wkly Rep* **53**, 362–365.
- Williams LJ, Rasmussen SA, Flores A, *et al.* (2005) Decline in the prevalence of spina bifida and anencephaly by race/ethnicity: 1995–2002. *Pediatrics* **116**, 580–586.
- De Wals P, Tairou F, Van Allen MI, *et al.* (2007) Reduction in neural-tube defects after folic acid fortification in Canada. *N Engl J Med* **357**, 135–142.
- Gucciardi E, Pietrusiak MA, Reynolds DL, *et al.* (2002) Incidence of neural tube defects in Ontario, 1986–1999. *CMAJ* **167**, 237–240.
- Moore LL, Bradlee ML, Singer MR, *et al.* (2003) Folate intake and the risk of neural tube defects: an estimation of dose-response. *Epidemiology* **14**, 200–205.
- Czeisel AE, Dobó M & Vargha P (2004) Hungarian cohort-controlled trial of periconceptional multivitamin supplementation shows a reduction in certain congenital abnormalities. *Birth Defects Res A Clin Mol Teratol* **70**, 853–861.
- Klusmann A, Heinrich B, Stoepler H, *et al.* (2005) A decreasing rate of neural tube defects following the recommendations for periconceptional folic acid supplementation. *Acta Paediatrica* **94**, 1538–1542.
- CDC (2008) Prevalence of neural tube defects and folic acid knowledge and consumption – Puerto Rico, 1996–2006. *MMWR Morb Mortal Wkly Rep* **57**, 10–13.
- Busby A, Armstrong B, Dolk H, *et al.* (2005) Preventing neural tube defects in Europe: a missed opportunity. *Reprod Toxicol* **20**, 393–402.
- Queisser-Luft A & Spranger J (2006) Congenital malformations. *Dtsch Aertzebl* **103**, 2462–2471.
- FDA (1993) Food labeling: health claims and labeling statements: folate and neural tube defects. *Fed Regist* **58**, 53254–53295.
- Yang QH, Carter HK, Mulinare J, *et al.* (2007) Race–ethnicity differences in folic acid intake in women of childbearing age in the United States after folic acid fortification: findings from the National Health and Nutrition Examination Survey, 2001–2002. *Am J Clin Nutr* **85**, 1409–1416.
- Choumenkovitch SF, Selhub J, Wilson PW, *et al.* (2002) Folic acid intake from fortification in United States exceeds predictions. *J Nutr* **132**, 2792–2798.
- Pfeiffer CM, Caudill SP, Gunter EW, *et al.* (2005) Biochemical indicators of B vitamin status in the US population after folic acid fortification: results from the National Health and Nutrition Examination Survey 1999–2000. *Am J Clin Nutr* **82**, 442–450.
- Dietrich M, Brown CJP & Block G (2005) The effect of folate fortification of cereal-grain products on blood folate status, dietary folate intake, and dietary folate sources among adult non-supplement users in the United States. *J Am Coll Nutr* **24**, 266–274.
- CDC (2007) Folate status in women of childbearing age, by race/ethnicity – United States, 1999–2000, 2001–2002, and 2003–2004. *MMWR Morb Mortal Wkly Rep* **55**, 1377–1380.
- Pfeiffer CM, Johnson CL, Jain RB, *et al.* (2007) Trends in blood folate and vitamin B₁₂ concentrations in the United States, 1988–2004. *Am J Clin Nutr* **86**, 718–727.
- Esfahani ST, Cogger EA & Caudill MA (2003) Heterogeneity in the prevalence of methylenetetrahydrofolate reductase gene polymorphisms in women of different ethnic groups. *J Am Diet Assoc* **103**, 200–207.
- Oakley GP (2007) When will we eliminate folic acid-preventable spina bifida? *Epidemiology* **18**, 367–368.

43. Johnston RB (2008) Will increasing folic acid in fortified grain products further reduce neural tube defects without causing harm? Consideration of the evidence. *Pediatr Res* **63**, 1–3.
44. Quinlivan EP & Gregory JF (2007) Reassessing folic acid consumption patterns in the United States (1999–2004): potential effect on neural tube defects and overexposure to folate. *Am J Clin Nutr* **86**, 1773–1779.
45. Botto LD, Lisi A, Robert-Gnansia E, *et al.* (2005) International retrospective cohort study of neural tube defects in relation to folic acid recommendations: are the recommendations working? *BMJ* **330**, 571.
46. Charles DH, Ness AR, Campbell D, *et al.* (2005) Folic acid supplements in pregnancy and birth outcome: re-analysis of a large randomised controlled trial and update of Cochrane review. *Paediatr Perinat Epidemiol* **19**, 112–124.
47. Canfield MA, Collins JS, Botto LD, *et al.* (2005) Changes in the birth prevalence of selected birth defects after grain fortification with folic acid in the United States: findings from a multi-state population-based study. *Birth Defects Res A Clin Mol Teratol* **73**, 679–689.
48. Johnson CY & Little J (2008) Folate intake, markers of folate status and oral clefts: is the evidence converging? *Int J Epidemiol* **26**, 1–18.
49. Lawrence M & Riddell L (2007) Mandatory fortification with folic acid – what would Hippocrates say? *Aust Fam Physician* **36**, 69–73.
50. Smith AD (2007) Folic acid fortification: the good, the bad, and the puzzle of vitamin B12. *Am J Clin Nutr* **85**, 3–5.
51. Smith AD, Young-In K & Refsum H (2008) Is folic acid good for everyone? *Am J Clin Nutr* **87**, 517–533.
52. Ulrich CM & Potter JD (2007) Folate and cancer – timing is everything. *JAMA* **297**, 2408–2409.
53. Mason JB, Dickstein A, Jacques PF, *et al.* (2007) A temporal association between folic acid fortification and an increase in colorectal cancer rates may be illuminating important biological principles: a hypothesis. *Cancer Epidemiol Biomarkers Prev* **16**, 1325–1329.
54. Morris MS, Jacques PF, Rosenberg IH, *et al.* (2007) Folate and vitamin B₁₂ status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification. *Am J Clin Nutr* **87**, 193–200.
55. Selhub J, Morris MS & Jacques PF (2007) In vitamin B12 deficiency, higher serum folate is associated with increased total homocysteine and methylmalonic acid concentrations. *PNAS* **104**, 19995–20000.
56. Bazzano LA, Reynolds K, Holder KN, *et al.* (2006) Effect of folic acid supplementation on risk of cardiovascular diseases: a meta-analysis of randomized controlled trials. *JAMA* **296**, 2720–2726.
57. Winkels RM, Brouwer IA, Clarke R, *et al.* (2008) Bread cofortified with folic acid and vitamin B-12 improves the folate and vitamin B-12 status of healthy older people: a randomized controlled trial. *Am J Clin Nutr* **88**, 348–355.