REVIEW

Folates for reduction of risk of neural tube defects: using oral contraceptives as a source of folate

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Abstract: The evidence that folates reduce the risk of neural tube defects (NTDs) is so compelling that supplementation has been recommended by every relevant authority. The Cochrane Database of Systematic Reviews has determined that folate supplementation should be rated as a Grade 1 recommendation. United States Preventive Health Services Task Force, the US Food and Drug Administration (FDA), and the Centers for Disease Control and Prevention (CDC) have all produced clear guidelines for such supplementation. Unfortunately, despite food fortification and targeted public health campaigns promoting folic acid supplementation, periconceptional utilization of folic acid supplements has decreased in the US in recent years. Worldwide, over 300,000 newborns are affected with NTDs every year. NTDs account for 10% of all neonatal mortality. This article will review the risk factors for NTDs and the evidence supporting folate supplementation. It will also describe the remaining problems and outline current ideas to solve them. Finally, new evidence of the effectiveness of adding metafolin to drospirenone-containing oral contraceptives in raising serum and red blood cell folate levels, the rationale for making such an addition, and an estimate of the magnitude of the contribution use of such pills might have on reducing NTDs will be discussed.

Keywords: neural tube defects, folate, metafolin, oral contraceptives

Introduction

Grains and cereals in the US have been fortified with folic acid for over a decade to reduce the risks of neural tube defects – so it is reasonable to ask: why do we need to provide any extra folate supplementation? It is also reasonable to ask: if women are using oral contraceptives to protect against pregnancy, why should we use those pills to reach women at risk for having pregnancies affected by neural tube defects?

In this article we will review the spectrum of neural tube defects (NTDs), the impact NTDs have on quality of life, what is known of their genetic causes, and the connection between NTDs and folates. We will also analyze the outcomes of the different public health interventions, which have been adopted over time to increase folate for reproductive age women and we will describe the remaining challenges. Finally, we will describe the latest approach to providing folate supplementation to women at risk for pregnancy – the addition of tetrahydrofolate to drospirenone-containing oral contraceptives.

Definitions of neural tube defects

Neural tube defects are congenital malformations that result from failure of the neural tube to close during embryogenesis. The primordial central nervous system starts as

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a plate of cells, which folds on itself to form a tube between days 21 and 28 postconception. Neural tube defects include spina bifida, anencephaly, encephalocele, craniorachischisis, and iniencephaly.[1] The type of NTD that develops depends upon the timing (and thus the location) of the defect. If the failure occurs at the cranial end, the resulting malformation is called anencephaly. Anencephalic fetuses lack variable amounts of brain and cranium above the base of the skull and the orbits. Defects at the cranial end of the neural tube, collectively called spina bifida, occur in the midline of the lumbosacral region. These defects involve dysplasia of the spinal cord, neural roots, and meninges. If the herniated sac contains neural elements, the defect is called a meningomyelocele; if only the sac protrudes, the defect is a meningocele. The defect may or may not be covered by skin. Encephalocele represents a postclosure NTD. It is a membrane-covered protrusion of the brain tissue through an abnormal opening in the skull, typically in the occipital area. Microcephaly and hydrocephalus are common findings associated with encephaloceles.[2] This paper will focus on the two main defects that have been linked with closure formation defects – anencephaly and spina bifida.

Incidence of NTDs

Neural tube defects are the most common malformations of the central nervous system and collectively comprise the second most common congenital anomaly overall.[3] Worldwide, spina bifida and anencephaly affect 300,000 newborns each year.[1,4] These defects are responsible annually for 41,000 deaths and 2.3 million disability-adjusted life years.[5] Neural tube defects alone account for 10% of total neonatal mortality[6] and comprise 10% of the burden of all congenital conditions. The incidence of NTD varies between 1 and 10 per 10,000 live births each year.[7] Prevalence differs by geographic area, socioeconomic status, and ethnicity. The areas of highest prevalence in Western countries are Ireland and Scotland, where the baseline prevalence is 10/1000 live births.[3] In the US, the greatest prevalence of NTDs is found in the southern states.

The birth rate of infants affected by NTDs has dropped in the last 30–40 years because of better prenatal diagnosis utilizing widespread serum alpha fetoprotein testing and high resolution ultrasound, coupled with selective abortion practices. Prenatal detection rates are reported to be in the range of 82%–98%.[8,9] Once an NTD is detected, these same studies report that 60%–83% of affected pregnancies are terminated. However, despite these antenatal screening programs, food fortification, and extensive public

health campaigns recommending preconceptional folic acid supplementation, 2500 infants are born in the US each year with NTDs[10] and another 1500 pregnancies affected by NTDs are terminated.[11,12] It is estimated that at this time about 30,000 Americans are living with spina bifida.[3]

NTDs represent a major health problem. The mortality rate for anencephaly is 100%; for infants affected by spina bifida, the mortality rate is about 30%. The survivors with spina bifida suffer significant morbidity from neurological defects that correspond to the level of their malformations. Most spina bifida results in paralysis in the lower extremities and loss of bladder and bowel function. Affected individuals usually require significant medical attention and experience long-term disabilities that affect their survival, functional abilities, and economic productivity. The development of intermittent bladder self-catheterization[13] has been recognized as providing major improvement in quality of life for those with spina bifida[1] but latex allergy rates are among the highest in this population and recurrent urinary tract infection can ultimately lead to renal failure.

NTD etiology

Neural tube defects have a heterogeneous and multifactorial etiology, including genetic and environmental factors as well as predisposing maternal factors.[14] Genetic predisposition is suspected because of the following: the prevalence of NTDs varies in different racial and ethnic groups,[15,16] the excessive numbers of affected females compared to males, the high rate of consanguinity seen in parents of NTD-affected infants, the association of NTDs with more than 80 genetic abnormalities (trisomy 13 and 18 and autosomal recessive syndromes, such as Meckel–Gruber)[17], and the high rates of recurrence in subsequent pregnancies.[14,18] Environmental maternal health influences are suspected because of wide geographic variations in incidence as well as the identification of other risk factors, such as nutrition, maternal diabetes (relative risk for NTDs of 15.5),[19] use of valproic acid,[20] hyperthermia,[21,22] obesity,[23–25] and occupational exposure to agricultural[26] and cleaning products.[27,28] In areas where a folate-rich diet is not readily available, the risk of NTDs is highest among the most economically disadvantaged.[29,30]

Genetic etiology of NTDs

Knowledge about the genetic etiology of NTDs is limited because the techniques used to identify individual gene loci require genetic information from multiple affected family members, which is a rare occurrence with NTDs. There are 200 small animal models of NTDs, but most do not replicate

the human disease phenotype. Reflecting known risk factors, over 100 candidate genes have been suggested for spina bifida, including those involved in folic acid metabolism, glucose metabolism, retinoid metabolism, and apoptosis, but fewer than 20% of the genes studied confer even minor risk association.[7] NTDs have been found to be closely related to genes that encode proteins that are directly or indirectly connected with folic acid and methionine metabolism. The most productive research has, therefore, focused on the variation in genes encoding for enzymes related to folate uptake and the metabolism of both folate and homocysteine.[14]

Genetic impacts on folate metabolism

Natural dietary folates are polyglutamates that must be deconjugated into monoglutamates before they can be absorbed in the proximal small intestine. Deconjugation is accomplished by the enzyme folylpoly-gamma-glutamate carboxypeptidase. The folate most commonly found in the bloodstream is 5-methyltetrahydrofolate monoglutamate (THF). THF can enter cells directly by means of high affinity receptors that are found in the proximal tubules of the kidney, the choroid plexus and, importantly, in the placenta. There is also a high density of folate carrier in the embryonic neural tube, supporting the critical role for folate during neural tube closure. Other lower affinity receptors are located on numerous sites. In addition to receptor-mediated transport, THF can enter cells by a carrier-medicated transport via reduced folate carrier (RFC), which is ubiquitously expressed. Once THF enters the cell, another glutamyl group is added so THF cannot be transported out of the cell.

Delivery of folate to the embryo depends not only on maternal intake, but on maternal absorption and metabolism, as well as folate transport across the placenta and embryonal folate uptake.[31] In either the mother or the embryo, mutations in genes in the folate cycle, such as methylenetetrahydrofolate reductase and folate receptor genes, cause NTDs.[32,33] The most significant genetic variations have been found in the genes encoding for enzymes of folate metabolism and the closely-related homocysteine metabolism.[14] The most important enzyme in the regulation of available folate is the enzyme methylenetetrahydrofolate reductase (MTHFR). A common mutation in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene has been identified as a risk factor for NTDs.[34,35] The MTHFR enzyme allows the cells to regulate intracellular concentration of methionine and homocysteine. One specific thermolabile variant, MTHFR C677T SNP, in which there is an alanine-to-valine substitution, has

been associated with a 66%–100% increase in the risk of NTDs in their mothers,[14,36] especially those with low folate levels.[37] This mutation has been reported to account for 26% of Irish NTDs.[38] This mutation causes the enzyme to lose its activity in the face of an increase in temperature, results in increases in homocysteine, and requires higher levels of folate to stabilize the binding of the constituent parts of the MTHFR enzyme.[59] This mutation is present in 4%–16% of healthy adults, with the highest prevalence in Japanese men.[39] A second SNP – the MTRR A66G SNP, which is involved in methionine synthetase reduction – has also been associated with a 48% increase in risk for NTDs.[14]

Because of altered folate-dependent metabolic activity, some segments of the population may require more folate than their local diet is capable of supplying.[47] They may also need higher amounts of supplementation to benefit from preconceptional supplementation. They would also benefit from using the most easily utilizable form of folate (L-5-methyltetrahydrofolate).

The connection between NTDs and folate: an epidemiologic success story

There are only a few instances in which science has been able to identify preventable causes of birth defects. The most prominent of these includes recognition of rubella virus as a cause of congenital rubella syndrome, cytomegalovirus as a cause of birth defects and developmental disabilities, alcohol as a cause of a spectrum of defects, and folic acid as preventive for neural tube defects, especially the two defects we are discussing, spina bifida and anencephaly.[40] A recent Cochrane review concluded that folic acid alone or in combination with vitamins and minerals prevents NTDs.[41] Oakley characterized the discovery that a simple cheap and effective public health intervention – giving a vitamin – could keep thousands of children alive each year and out of wheelchairs as a "humanistic miracle".[42] Indeed the folate story is quite impressive. Folate pioneers had to overcome significant barriers on their path to discovery of that relationship and even more barriers to introduce the measures needed to prevent these birth defects.[43–45]

The relationship between NTDs (especially anencephaly and spina bifida) and folate deficiency was first suggested in 1964.[46] It was not an easy association to recognize because most women who deliver babies with NTDs have blood levels of folate that are within normal limits as defined for the general public.[47] One early study indicated that levels of abnormal formiminoglutamic acid excretion test were five-fold higher in women who delivered infants with NTDs than those

who delivered normal infants, when the women were tested around the time of delivery. However, the authors concluded that that abnormality might indicate defective absorption or metabolism rather than a deficient intake of folate.[48] On the other hand, Smithells hypothesized that the cause of NTDs might be nutritional, because poor women had a higher risk than better fed, wealthier women.[44] Unfortunately, three of four hospitals in which Smithells sought to test his hypothesis refused to allow a randomized trial, so the power of his findings of a reduction in NTDs with folic acid supplementation in a nonrandomized trial were less persuasive than might have been expected for such an important breakthrough.[42]

The most convincing evidence of the association between NTDs and folic acid came from women who had experienced previous pregnancies affected by NTDs. The recurrence rate for NTDs is about 2.9% in North America and 4% in the British Isles, but it can be as high as 10% in other areas.[49] The landmark study by the Medical Research Council Vitamin Study Group was a randomized trial of folic acid (4 mg/day) vs placebo for the prevention of recurrent NTDs. When the 4 mg dose was started at least 1 month before conception and was continued through the first trimester, a 72% reduction in NTDs was seen in the intent-to-treat population and a 83% reduction was seen among the per-protocol women.[43] A later meta-analysis of four randomized trials of folic acid for the prevention of recurrent NTDs showed a 69% reduction in an intent-to-treat analysis and an 87% reduction in the risk of recurrence among women who started treatment prior to pregnancy.[49] Studies with lower doses of folic acid (0.35 mg) and less frequent doses failed to find benefit.[50] Unfortunately, the percentage of women with prior affected pregnancies who actually take *any* folic acid supplements before subsequent pregnancies has been reported to vary between 33% and 85%.[49]

Although studies of women with prior NTD-affected pregnancies persuasively demonstrated the protection folate provided, 90% of cases of NTDs occur in women who have never experienced a NTD-affected pregnancy. Since it is not clear that the defect mechanisms would be the same in those two different populations, it was important to also study interventions in the general population. Between 1988 and 1995, five case-controlled studies of women without prior pregnancies affected by NTDs (spina bifida or anencephaly) investigated the impact that preconceptional folate supplements with or without multivitamins and food folate would have on the incidence of NTDs.[51–55]

Only one study with all three interventions in the study arm failed to find any decrease in NTD incidence.[53] The other four studies reported reductions in the risk of NTDs

in the range of 35%–75%.[51,52,54,55] A later large cohort study in China also demonstrated the effectiveness of folic acid supplementation in preventing NTD in first pregnancies; the reductions seen in this study also showed geographic variation with protection ranging from 41% to 79%.[56] One prospective, but nonrandomized, cohort study with dietary folate and a folic-acid-containing multivitamin studied in 22,000 women found a mean reduction in NTDs of 71% among women who started the supplement at least 1 month prior to conception and continued to take it through the first trimester.[57] The only randomized prospective trial of folic acid compared folic acid (0.8 mg) with multivitamins to a supplement containing trace elements. In that study, no cases of NTDs occurred in the 2104 women in the folic acid arm, but six cases were seen in the 2052 women who were given trace element supplements.[45]

Today it is estimated that 50%–60% of NTDs could be prevented by adequate periconceptional folic acid.[3] Some experts have estimated that the lowest levels of NTDs to be expected with folate supplementation would be four to five cases per 10,000 births or seven to eight cases at birth or abortion per 10,000 births.[58]

Definition of folates

"Folate" is a generic term for a water soluble B-complex vitamin, which serves as an enzyme in transfer of single carbons during the synthesis of the purines and pyrimidines that are required for DNA synthesis and repair.[3] The folate cycle is also involved in another essential physiological process: the methylation associated with the methionine cycle, which is particularly important during the initial period of embryonic development.[59] Folate helps produce and maintain new cells, especially at a time of rapid cell division and growth. Low folate levels are also associated with megaloblastic anemia.

Folates occur in foods as polyglutamates. Foods that are good sources of dietary folate polyglutamates include spinach, lentils, chickpeas, asparagus, broccoli, peas, brussel sprouts, nuts, corn, and oranges as well as kidney beans (Table 1). Polyglutamates must be unconjugated to form monoglutamates before they can be absorbed. Folic acid is rarely found in food but is an oxidized monoglutamic acid (pteroylglutamic acid) that is the most active form of the vitamin. Polyglutamates from foods have only about half the absorbance of folic acid and have even less bioavailability on an empty stomach.[3] Therefore, folic acid is the form used both in vitamin supplementation and in food fortification.

Table 1 Food sources of folic acid[145]

Food source	Amount of folic acid (mcg/100 g* of food)
Dark green leafy vegetables	120–160
Other vegetables	40–100
Citrus	50–100
Beans (legumes)	50–300
Whole grains	60–120
Breakfast cereals (fortified)	100–400

Note: *100 g = 3.5 oz.

Folate requirements

Most fetal organogenesis is established within the first few months of pregnancy. A mother's nutritional status preconceptionally can have lifelong consequences for the child. It is clear that it is not adequate for pregnant women to consume a diet that only avoids severe nutritional deficiencies; women need diets that provide sufficient nutrients of all types to optimize fetal anatomic and neurologic development.[60] Both excesses of certain nutrients (eg, glucose) and deficiencies in others (folate, protein, iron, vitamin B6, calcium, zinc) can cause deleterious changes in physiologic set points, organ size, and cell-signaling programming. Timing of these dietary changes is important; in particular, it is important that the intake of folates be increased prior to conception. Good nutritional assessment and counseling is a critical component of preconceptional care.[60]

The Institute of Medicine recommends consumption of folic acid 400 mcg/day for adult women (age 19 and over) and 800 mcg/day for women aged 14–18 years.[61] The average amount of folic acid received through fortification from grains in the US is only 128 mcg/day, so supplementation is often needed.[62] Even folic acid requires metabolism to activate it. Genetic influences in folate absorption and metabolism can occur at several levels and may limit the usefulness of supplementation for some individuals.

Historic approaches to enhancing folate levels prior to pregnancy

At least 3 months of folic acid supplementation is needed to achieve adequate steady-state folate levels.[60] Concentrations of folate in red blood cells less than 906 nmol/L are associated with higher than baseline risk for NTDs.[63] Folic acid supplementation that is not initiated until the first prenatal visit is too late to prevent NTDs; the malformation may already be a reality. Classically, there have been three approaches tried in the US to ensure that women have adequate levels of folate prior to organogenesis.[64] These approaches vary in their target audiences as well as in their recommended interventions.

The CDC initially recommended that the target population for folic acid supplementation should be all reproductive-aged women.[65] The Institute of Medicine (IOM) narrowed the target population to "women capable of becoming pregnant". In the assessment of the IOM, fewer than half of US women age 15–44 are at risk of pregnancy. This is because 22% of US women are permanently sterilized, 20% are using a "highly effective method", 11% have never had coitus, and 5% are pregnant or recently delivered. The US Preventive Health Services Task Force recommends that all women planning or capable of pregnancy take a daily supplement containing 0.4–0.8 mg (400–800 mcg) of folic acid. They labeled the recommendation as Grade A.[66] The two approaches that have been tried over the years to increase periconceptional folate levels in women include:

- Recommend folic acid supplementation for the target population.
- Increase folic acid intake by fortifying food supplies so that all women will automatically be provided adequate levels.

These approaches may be complementary.

Folic acid supplements

Early efforts to target folic acid supplementation to women planning pregnancy were useful,[64] but only had a modest impact.[67] In fact, the frequency of NTDs in the US did not decline following extensive lay and professional educational campaigns about folic acid supplementation.[68] Several problems with targeted supplementation have been identified. The first problem identified was that women were not aware of the need to consume extra folate. A survey in 1998 indicated that only 29% of US women said they were following the recommendation that they consume 400 mg of folic acid daily.[69] In another survey, even among women who knew of the need for folic acid, knowledge did not translate into action. Following the campaigns, folic acid utilization rose overall from 14% to 23%.[70] In 2007, the March of Dimes found in a national survey in the US that 36% of women surveyed did not even know the meaning of the term "preconceptional care".[71] International studies have also reported infrequent preconception use of folic acid supplement.[72–74] A recent study in Israel found that only 7.7% of Jewish women and 3.1% of Bedouin women used folic acid for 3 months before conception.[75] Secondly, most pregnancies in the US are not planned and prepared for, and we do not even have statistics estimating rates of planned pregnancies. All that is measured is "unintended" pregnancy. Similarly, a recent special report from the European Surveillance on Congenital

Anomalies group noted that in Europe, many pregnancies are unplanned.[76] The other major problem with targeted supplementation was that clinicians did not routinely screen women and educate them on the need for preconceptional folic acid supplementation.[77,78]

The bottom line was seen in a meta-analysis of eleven population-based registries and 15 surveillance systems from Europe, North America, and Australia, which found no significant changes in trends for NTDs in areas with supplementation recommendations alone.[77] Another international study of over 13 million births found that the incidence rates of NTDs were unchanged by the supplement campaigns.[1] A Swiss study revealed recently that only 5% of women in that country correctly took periconceptional folic acid.[79] In fact, one newer study found that only 20% of Dutch women in lower socioeconomic status groups knew the correct period of use of folic acid. The study was conducted 8 years after a mass media campaign. The authors concluded the once-only campaign had only a short-term effect.[80]

Food fortification

Given the obvious failure of voluntary targeted folic acid supplementation, attention turned to food fortification. The topic was controversial because there were issues of costs and potential health risks from fortification.

Fortunately, fortification of specific frequently consumed food stuffs, such as cornmeal and wheat bread, does not affect their apparent taste or shelf life. In a benefit–cost analysis performed prior to the FDA mandate, the unit cost of folic acid was estimated to be US$0.0095/100 g of grain. The cost of testing to demonstrate appropriate levels in the final product was another $2.5 million. Labeling changes were expected to be the most costly item, totaling $20 million. On the benefits side, the cost per child with spina bifida was assumed to be nearly $350,000. Given these costs and benefits, the authors estimate a net annual benefit of $121 million and a benefit-to-cost ratio of 4.3 to 1.[81]

Beyond these economic costs, there was concern about adverse health effects that the addition of folic acid might have on vulnerable populations, such as the elderly and vegetarians. The most concerning impact would be that folic acid supplements would mask megaloblastic anemia due to vitamin B_{12} deficiency, ie, that folic acid would induce laboratory normalization while there was progression of neurological manifestations of pernicious anemia, including cognitive impairment, subacute degeneration of the spinal cord or other neurological sequelae.[82] Another possible adverse effect of increasing folates in the diet would be experienced

by individuals using medications with intentional antifolate effects, such as methotrexate, pyrimethamine, trimethoprim, triamterene, and sulfasalazine. There was also concern that additional dietary folate might affect the absorption or metabolism of trace minerals, especially zinc, although decreased zinc excretion should balance that effect. The lack of data about long-term safety of continuous high intake of folate also troubled some people.[83]

Despite these concerns, the benefits expected from fortification outweighed those risks. The US Food and Drug Administration mandated that by 1998 all enriched grain products would be fortified with 140 mcg folic acid per 100 g of grain.[84] This level of enrichment was expected to raise US consumption of folate by about 100 mcg a day. At folate levels achieved by the addition of 100 mcg of folic acid, it was expected that the percentage of US women who would consume the minimum recommended amount of folic acid a day (400 mg) would increase from 29% to 50%.[43,85,86]

Follow-up studies found that blood folate levels in the general adult population increased significantly following folic acid fortification of enriched grain products,[87–91] and that there was a measurable decline in neural tube defects after food fortification.[92–96] These studies noted a 21%–34% reduction in the occurrence of spina bifida and an 11%–20% reduction in anencephaly. Reductions were higher in Canada (53% reduction in spina bifida and 38% reduction in anencephaly)[97] and in Chile (51% lower rates of spina bifida and 45% lower rates of anencephaly).[98] The gap identified between the results seen with fortification experienced in the US and those seen in other countries raised many questions. It may have been that US women were already more folate-replete than other populations or there may have been other explanations.

The first answers to this question came from follow-up done by the National Health and Nutrition Examination Survey (NHANES III), which revealed that the proportion of women in the US aged 15–44 years who achieved therapeutic levels following fortification increased by only 33%, not the expected 50%.[85] In fact, the FDA's goal to have 50% of women of childbearing age consume 400 mcg folic acid was not reached by any racial or ethnic group in the US following food fortification. The percentage of women who consumed the desired amount after fortification varied. For Caucasian women, the percentage of women with adequate intake (400 mcg/day) rose from 30% to 39%; for African-American women, it rose from 20% to 26%; and for Hispanic women, the percentage change went from 17% to 28%. A lower goal of 200 mcg/day was met by about 90% of Caucasian and Hispanic women and by 70% of African-American

women. Interestingly, NHANES data also found that after food fortification, the proportion of people taking folic acid supplements actually declined.[85] Although with food fortification, serum folate levels initially increased, some of those initial gains were lost over time. The CDC found that serum and red blood cell folate levels in nonpregnant reproductive age women decreased significantly from 2000 to 2004, by 16% and 8%, respectively.[99] As a result of this shortfall in increase in folate levels, NTD rates in the US decreased by only 20%–38% following fortification,[12,96,100–102] not the 50%–70% reduction predicted.[57]

Several hypotheses have been advanced for this decline in folate levels, including a decline in the use of folic acid supplements, a decline in the consumption of fortified foods or foods rich in folates, and an increase in medical conditions, such as obesity, that affect blood folate levels.[103] Folate requirements may be higher in obese women due to abnormalities in the release of that vitamin from body stores.[23,104] A Gallup Poll in 2007 found that the groups least likely to use folic acid supplements were young women (aged 18–24), less educated, and less affluent women.[71] Unfortunately, over half of reproductive age women in that study reported that they had never been advised by a health care provider to take a multivitamin.

This shortfall in NTD reduction from the promise offered by food fortification is of particular concern for low income women, who are known to have 16%–24% lower serum folate levels than more affluent women.[105] These same women are also at higher risk for having suboptimal folate levels because of lower use of supplements.[106] Over half (58%–63%) of low income reproductive age women in California did not follow the recommendations for folic acid supplementation.[107] Knowledge of the benefits of folic acid supplements is also low among low income women. Both serum and red blood cell folate levels for women of childbearing age are lowest among African-American women and highest among White women, with Hispanics in between. Birth certificates, which provided information on live births in 45 US states and in the District of Columbia, found that birth prevalence of NTDs decreased from 37.8 per 100,000 live births before food fortification to 30.5 per 100,000 live births conceived after mandatory folic acid fortification. This represented only a 19% decline (prevalence ratio = 0.81; 95% confidence interval [CI]: 0.75–0.87). This same report found that during the same time period, NTD birth prevalence for women who received only third trimester or no prenatal care declined by almost the same percentage from 53.4 per 100,000 to 46.5 per 100,000 with a prevalence ratio of 0.87 (95% CI: 0.64–1.18).[92] The

authors concluded that since the late prenatal care group had a decline of about the same magnitude as the overall group, both those improvements must be due to food fortification because the 13% decline observed in those with no or late prenatal heath care could not be due to significant increases in early diagnosis and pregnancy termination.

Possible solutions to the remaining problem
Increased fortification
Some have suggested that to further reduce NTDs, we need to increase the levels of food fortification.[108,109] Since the original FDA mandate, many groups have requested that the supplement be raised from 140 mcg/100 g to 350 mcg/100 g.[11] Some experts have suggested that the threat to vulnerable populations would not become clinically significant until fortification levels raised the daily intake of folic acid to 5 mg or more.[81,110] One small study compared folate levels in women randomized to two different folic acid doses: either 1.1 mg or 5 mg daily for 30 weeks. Red blood cell folate levels were 14.4 times higher with the 5 mg dose and therapeutic levels were achieved earlier.[111] In another approach, investigators in Honduras found that higher doses of folic acid (5 mg) given less frequently (weekly) did not increase blood folate levels more rapidly than a daily dose of 1 mg folic acid.[112] Others have argued that the concern over pernicious anemia could be addressed directly by adding vitamin B_{12} as a second ingredient in the fortification (co-fortification) of the grains and cereals.[113] In a 12-week study, men and women aged 50–75 years were randomized to receive bread fortified with 130 mcg folic acid and 9.6 mcg vitamin B_{12} daily or unfortified bread. In those who received the vitamin supplements, both folate and vitamin B12 levels rose by 45%. All subjects in the active arm with borderline low vitamin B_{12} levels normalized their vitamin B_{12} levels.[113]

Recently a new concern has been raised about folic acid fortification – the potential for an increased risk of cancer.[114] Theoretically, folic acid supplementation could enhance DNA replication and increase the rate of growth of pre-existing carcinoma. Analysis of two recent randomized, double-blind, placebo-controlled clinical trials studied the impact of homocysteine-lowering drugs (folate and vitamin B_{12}) on cardiovascular morbidity and mortality in patients with ischemic heart disease. In a post-hoc analysis, it was found that during the trial and the 3-year follow-up period, both the incidence and mortality of newly diagnosed "cancer" was higher in the folic acid supplementation arms. The hazard ratio was 1.21 and 1.12 for each of these outcomes.

The number of cases was too small to find a statistically significant risk for any of the very disparate cancers studied. Of note, the study was done in Norway, where there is no folate food fortification.[115] The authors point out that other investigators have not demonstrated any association between folate or folic acid and the risk of cancer of the lung[116] or colon or rectum.[117,118] North American studies of folic acid supplements also do not support these findings.[119,120]

The final argument against increasing the amount of folic acid fortification is that it will yield diminishing returns because an increasing proportion of the remaining cases of NTDs are not folate responsive. Not everyone agrees; one theoretical model predicted that folic acid 0.4 mg would reduce NTDs by 36% but 5 mg folic acid would reduce NTDs by 85%.[121] However, this model has never been tested clinically. To expose the entire population to higher folate levels to obtain smaller and smaller reductions in NTDs may not be good public policy. Other approaches may be needed for the remaining women at risk.

Increase efforts at targeted supplementation

Although significant progress has been made since 1995 in educating women about the importance of taking a vitamin with folic acid, only 12% of women in the 2007 survey knew that folic acid should be taken daily before pregnancy to reduce the risk of birth defects.[71] Experts have suggested that the failures of prior efforts at providing public education about the need for reproductive age women to use supplemental folic acid were due to the approaches used.[103] Learner-centered approaches to adult nutritional education provide targeted messages using a wide variety of teaching styles to match the variety of learning styles seen among adults.[122] However, the investigators in these low income test populations found that their own interventions resulted in only a modest increase in folate intake.[107]

Unfortunately, shortly after food fortification was initiated in the US, surveys continued to demonstrate that even pregnant women were unaware of the importance of preconceptional use of folic acid. In one survey in Arizona, only 7.9% of patients were aware of the specific association between folic acid and neural tube defects.[123] This same study verified that, despite many public health messages to many communities in the state, only 38% of Spanish speaking prenatal patients were taking a daily multivitamin.

In North Carolina, a public health initiative was launched to see if providing women with free bottles of multivitamins and education would enhance usage. Only 25% of women reported vitamin use at baseline, but with the free vitamins, that percentage rose to 53%. Latinas experienced the largest increase in vitamin initiation, rising from 21% to 70%. Unfortunately, the program was later defunded by the State legislature.[124]

Given that clinicians have little time to counsel women about folic acid themselves, one unique trial tested a 15-minute computerized educational session combined with 200 free tablets to see if knowledge about and use of folic acid would increase. Follow-up interviews 6 months later found that women who got the education intervention were more likely to know that folate prevents birth defects than control women (46% vs 22%) and to know that its use is important in early pregnancy (36% vs 17%).[125]

At a minimum, clinicians should alert women who are using medications that are known to reduce folate levels of their increased risks for NTDs and of their need for pre-conceptional adjustments. It may be appropriate in at-risk women to assess their red blood cell folate levels to determine supplementation needs.[63] Women with health problems such as insulin-dependent diabetes, seizure disorders, obesity, family histories of NTDs, and high-risk ethnic groups may also need extra supplementation or longer times to prepare for pregnancy.[110]

Why add folate to oral contraceptives?

Despite all of our current efforts, estimates are that only 10% of the preventable spina bifida and anencephaly are actually being prevented.[126] Understanding that most women do not get adequate amounts of folate in their diets and do not seek preconceptional care, it is obvious that newer approaches are needed to reach women at risk for pregnancy. Oral contraceptive pill users may seem an unusual group to consider for targeting additional folate supplements, but there are several reasons to consider them. First, contraceptive users are clearly sexually active. Oral contraceptives are the most popular method of birth control, especially for younger reproductive age women (under the age of 35 years), the age group with the greatest number of pregnancies – both intended and unintended. For young women who stop taking the pill to become pregnant, very few start folic acid supplements before they discontinue the pill. Estimates are that 21.6% of them will conceive the first month following pill cessation unless they adopt another effective method.[127] Even if they started folic acid supplements as soon as they stopped their pills, 46.7% would be pregnant within the first 3 months, before they raised their serum folate levels to the protective range.[127] Very importantly, folate supplements are

also needed to prevent NTDs in the unintended pregnancies that occur while the woman is taking the pill and is certainly not prepared for pregnancy. Estimates are that each year, 1 million women who considered themselves to be "using the pill" become pregnant – these failures account for one out of every six pregnancies in the US.[128]

Very few of these pill failures occur to women who use the pill correctly and consistently. Clinical trials for virtually every pill demonstrate that the failure rate with correct and consistent pill use is less than 1%. However, when used in the real world where women experience barriers to access (most women are dispensed only one pack of pills at a time), have concerns about pill side effects or safety, and have strong ambivalence about pregnancy,[129] consistent pill use is relatively rare. In one study of over 1.7 million new start hormonal contraceptive users, less than one-third of women refilled their prescriptions "on time" (within 14 days of the next scheduled refill date) for 12 months.[130] The rest either completely discontinued or significantly interrupted their pill use. In two other studies, 30% of women completely discontinued their pills by 3 months.[131,132] International studies show that of the women who stop their pills, only 35% switch to another modern, effective method within 3 months.[133] In a more recent US study in which women were provided effective contraception for free, only 30% obtained a median of ten refills on time.[134] These findings were substantiated by a 12-month study in family planning clinics which reported that the 12-month continuation rate with pills was only 32.7 per 100 women years.[135]

Even when women do successfully gain possession of their pill packs, they often do not use them correctly and consistently. A retrospective study of 1311 women presenting for their first family planning visit in a public health department found that only 42% of users said they took their pills daily and only 20% claimed they took a pill within 2 hours of the same time each day.[136] This bleak assessment has been confirmed by a study in which computer chips embedded in the pill packs recorded the date and time pills were removed from the package. The researchers found that over 50% of established pill users missed three or more pills each cycle.[137] Most women who discontinue pill use do not adopt another method immediately and if they do use a new method, it is generally one with a higher failure rate.[138]

In the face of these realities, it seems prudent to add a folate supplement to birth control pills. If a folate supplement were added to pills, it could raise plasma and red blood cell levels to reduce the risk of neural tube defects both during times of pill interruption and for several weeks after pill discontinuation. As a

safety net, it could provide needed elevated folate levels until such time as the woman adopts another effective contraceptive method or she initiates folic acid supplementation. This concept was unanimously endorsed at the FDA Health Drugs meeting in 2003.[139] In a small study to test the FDA recommendation, it was found that short-term use (8 weeks) of 500 mcg folic acid per day raised serum folate levels and that those levels did not return to baseline for 12 weeks following pill cessation.[140]

New products

There are two drospirenone-containing oral contraceptives that have added a very efficient form of folate in doses large enough to reliably raise plasma and red blood levels. The folate that was selected, metafolin, is levomefolate calcium, which is a synthetic calcium salt that is structurally identical to L-5-methyltetrahydrofolate (L-5-methyl-THF). As noted earlier, L-5-methyl-THF is the predominant folate transport seen in the blood with folic acid supplementation as well as with dietary folate. Tetrahydrofolate is directly absorbed from the intestine and requires no further processing before it can enter red blood cells. Genetic differences do not reduce the amount of folate that the body can utilize when tetrahydrofolate is used as the source of folate.

The predictability of metafolin-enriched drospirenone-containing oral contraceptives was demonstrated in clinical trials used as the basis of FDA approval. In one trial, women who ate a folic acid-fortified diet (the US diet) were assigned to receive either the original 24/4 birth control pill with 20 mcg ethinyl estradiol and 3 mg drospirenone or the new formulation which added 0.45 1 mg metafolin to each pill.[141,142] Folate levels were assessed in the plasma and red blood cells every 2 weeks for the 24-week study period (see Table 2). The women using the metafolin-supplemented oral contraceptives demonstrated significant increase (15.8 nmol/L) in serum folate levels. These levels were consistently maintained throughout the study period. In the conventional pill arm, plasma folate levels decreased by 2.2 nmol/L. Significant differences were also seen in the mean red blood cell folate levels. In the control group, red blood cell folate levels

Table 2 Plasma folate and RBC folate baseline values vs value following 24-week therapy 20 mcg EE/DRSP vs levomefolate calcium[142]

	EE/DRSP/levomefolate calcium		EE/DRSP		
N	Baseline	24 weeks	N	Baseline	24 Weeks
Plasma folate	196	45 ± 17.6	66	43.1 ± 16.1	41.0 ± 17.0
RBC folate	124	990 ± 390	45	1014 ± 308	1024 ± 293

Abbreviations: DRSP, drospirenone; EE, ethinyl estradiol; RBC, red blood cell.

increased by 34.3 nmol/L, while the metafolin-supplemented pill users demonstrated a mean increase from baseline of 419.9 nmol/L ($P < 0.001$).[141,143] While the P values in this study were impressive, the FDA advises that they be should be used with caution because a substantial percentage of data was dropped from each arm due to blood sampling errors.

In the second trial, German women, who do not have folate food fortification, were randomized into two arms in a double-blind, double-dummy trial. Women in one arm were given the monophasic formulation with 30 mcg ethinyl estradiol and 3 mg drospirenone with 451 mcg levomefolate calcium added to each of the 21 active pills and to each of the seven placebo pills and a placebo pill as a second tablet to take daily. In the second arm, women were given the same 30 mcg ethinyl estradiol, 3 mg drospirenone pill without metafolin and a tablet with folic acid 0.4 mg to take each day. This was a 24-week trial of drug use followed by an open-label follow-up 20-week period in which women received only the unfortified pill. At each point in time, the metafolin-treated women had higher levels of folate in both serum and red blood cells than women who took the folic acid supplements. Folate levels returned to baseline at about 20 weeks in the plasma, but were still above baseline levels in the red blood cells at the end of the 20-week follow-up period. Table 3 demonstrates the percentage of subjects who remained at or above protective levels of folate in the red blood cells (906 nmol/L) by weeks following cessation of metafolin (trial weeks 24–44).[139] Noninferiority was established by these two trials and the persistence of the elevation in folate levels was quantified.

Table 3 Kaplan–Meier estimates and the proportion of subjects for the time to RBC folate falling below 906 nmol/L for the yasmin + metafolin treatment group in the folate elimination phase (week 26 to week 44)[145]

Week	Number of subjects with RBC folate ≥906 nmol/L (n = 71)	Kaplan–Meier estimate (proportion of subjects with RBC folate ≥906 nmol/L)
24	71	0.947
26	70	0.933
28	64	0.853
30	59	0.787
32	45	0.600
34	35	0.467
36	22	0.293
38	18	0.240
40	13	0.173
42	10	0.133
44	7	0.093

Abbreviation: RBC, red blood cell.

A recent report sought to estimate the impact that switching all oral contraceptive users in the US to metafolin-containing formulations might have on the incidence of anencephaly and spina bifida. The model predicted pregnancy outcomes (live births, stillbirth, and elective terminations) after 6 months of use of the fortified pills. The authors estimated accidental pregnancies among pill users would occur in about 5%–8% of users and they assumed that 23% of women on the pills would discontinue pill use. Both of these estimates are conservative compared to many of the studies cited above. Based on these assumptions and the patterns of folate measured in the clinical trials of these metafolin-containing pills, the authors concluded that the number of NTD cases would decline by 23.7% to 31.4%, depending on the baseline folate levels of women switching from traditional birth control pills.[144]

Conclusion

Neural tube defects affect 300,000 pregnancies worldwide each year. Estimates are that 50%–60% of these defects could be prevented by achieving and maintaining adequate folate level periconceptionally. Recent research suggests another benefit of maternal use of folic acid periconceptionally (from 4 weeks before to 8 weeks after conception) – a reduced rate of severe language delay in children at age 3 years.[146] Food fortification with folic acid has raised serum and red blood cell folate levels and reduced neural tube defects, but not as impressively as predicted. Targeted supplementation with dietary recommendations and vitamins has not reached the population at risk; in fact, supplementation seems to have declined in the US over the last decade. At the same time, risk factors for neural tube defects, especially those related to maternal conditions, such as obesity and diabetes, have increased. In a novel approach to reach at-risk women, first approved by an FDA committee in 2003, a more uniformly utilizable form of folic acid L-5-methyltetrahydrofolate (metafolin) has been added to two birth control pills containing drospirenone. Studies have shown that the amount of metafolin in the pills is sufficient to raise both serum and red blood cell folate significantly. Further studies have demonstrated that therapeutic levels (sufficient to prevent folate-dependent NTDs) persist in the red blood cells of half the users for about 10 weeks after pill cessation and that all women maintained folate levels above their baseline for 24 weeks.

This legacy effect provides protection during vulnerable times – when women temporarily interrupt pill use and when women discontinue pill use. Both of those events occur with

surprising frequency, and help explain why 9% of pill users get pregnant in first year of pill use and why 1 million US "pill users" get pregnant each year. The addition of folate to pills offers the potential to reduce pregnancies affected by NTDs in these at-risk women.

Disclosure

The author serves on the advisory board for Bayer Healthcare for both the LNG IUS and Bayer's oral contraceptive products. Her clinic has received research grants from Bayer Healthcare and she has been active on Bayer's Speaker's Bureau.

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