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Folate and Prevention of Neural Tube Disease

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1. Introduction

A birth defect also referred to as "congenital anomalies" or "congenital abnormalities" is a health problem or a physical abnormality that a baby has at birth. It can be very mild or severe. Congenital malformations occur all over the world and are responsible for about 15% of the prenatal mortality in India (Merchant, 1989; Kulshteshtra et al., 1983; Swain et al., 1994; Datta & Chaturvedi, 2000). The relative importance of congenital malformations *per se* has snowballed as they manifest into a major public health concern owing to associated problems mortality, morbidity, social cost, and human suffering.

Among the various birth defects Neural Tube Defects (NTDs) are the most common malformations of the central nervous system that occur because of a defect in the neurulation process (Finnell et al., 2003; Sadler, 2005). Any woman of child-bearing age is potentially prone to give birth to a child suffering from an NTD. There are no means to predict the susceptibility of a woman to a NTD pregnancy as 95% of NTDs affect women without any history of such ailment in their families. Some affected pregnancies are spontaneously or electively aborted. Jaquier et al., 2006). Distinct variability has been reported in the occurrence rates for NTDs according to geographic area, socioeconomic status, and ethnic background (Frey & Hauser, 2003; Nazer et al., 2001; Pitkin, 2007; Benton, 2008).

The main cause of NTDs is yet to be known. They can be attributed to a combination of environmental and genetic factors (Sadler 2005). Among the various factors, nutrition has particularly been a significant factor effecting intrauterine development of fetus. Most of the attention has focused on folic acid as several research studies have shown that maternal folic acid supplementation reduce NTD incidence in humans ranging from 30-40% reduction in the general population to 70% for women given high levels of folic acid following a previous NTD pregnancy (MRC Vitamin Study Research Group,1991; de Wals et al., 2007). Folic Acid is a B group vitamin which plays an essential role in cellular division. Folate is required by human body to synthesize, repair and methylate DNA as well as to act as a cofactor in biological reactions involving folate (Goh & Koren, 2008). It s present ensures prolific cell division and growth, such as in infancy and pregnancy (Iyer & Tomar, 2009; Iyer et al., 2011) when the folic acid requirements are higher than usual. In consequence to the health benefits associated with increased folate intakes many countries now possess mandatory folate enrichment programs. The dose of folic acid, either alone or as part of a multivitamin preparation, varied between 400 to 5000 µg (0.4-5 mg) per day and was taken at least 1 month before conception and throughout the first trimester. In some countries, such as the

United States, Chile, Canada, and Israel, the food supply (usually flour) is fortified with folic acid as a way of bringing folic acid to women of childbearing age (Penchaszadeh, 2002).

Although folic acid supplementation is recognized to extend benefits for mothers-to-be, yet necessity of putting folic acid in the food supply is debatable (Iyer & Tomar, 2011). There has been discussion about the long-term effects of food fortification, as well as what effects folic acid may have on the general population, who would also be consuming the fortified foods (Yang et al., 2010). Lately, a number of studies have shown in comparison to natural folate, high intakes of folic acid, the chemically synthesized form (tolerable upper intake level, 1000 μg d^{-1}), can cause adverse health effects as highlighted by the Food and Drug Administration (FDA, 1996) such as the masking of the early hematological manifestations of vitamin B12 deficiency, leukemia, arthritis, bowel cancer and ectopic pregnancies. The Institute of Medicine (IOM, 1998) has established a tolerable upper intake level (UL) for folate from fortified foods or supplements (i.e. folic acid) for ages one and above. Intakes above this level increase the risk of adverse health effects. Therefore, naturally produced folates seem to be more rationale for fortification purposes. This article describes in the factors causing NTDs, role of folic acid, its side effects, advantage of natural folate over synthetic folic acid, need for novel food variants and further research needs.

2. Neural tube disease

NTD is a congenital malformation which takes place between the 20th and 28th day after conception (Sadler, 2005; Marco et al., 2011). It is an opening in the spinal cord or brain that occurs very early in human development. The cells of the neural plate make up the fetus' nervous system which during normal development folds back onto themselves to create the neural tube, which then becomes the back bone and the spinal cord and ultimately the brain. In the case of an NTD, the neural tube is unable to close completely and hence the brain and spinal cord remains exposed (Botto et al., 1999).

2.1 Types of NTDs

NTDs can be classified, based on embryological considerations and the presence or absence of exposed neural tissue, as open or closed types (Wald et al., 1991; Van Der Put et al., 2001; Greene et al., 2009a). Open NTDs are the most common which occurs due to a d defect in the skull or vertebrae and leads to exposed brain and/or spinal cord. Examples of open NTDs are spina bifida (myelomeningocele), anencephaly, and encephalocele. Closed NTDs are the rarer forms in which the spinal defect is covered by skin. Examples of closed NTDs are lipomyelomeningocele, lipomeningocele, and tethered cord. Anencephaly and spina bifida are the most common NTDs and occur with about equal frequency, whereas encephalocele is seen less frequently (Brody & Shane 2001).

Anencephaly is the cranial defect in which the cerebral cortex and overlying bony calvarium fail to develop. Infants with this disorder are born without a scalp or cerebellum. It is invariably lethal, with death either before or shortly after birth (Jaquier 2006). In spina bifida (about two-thirds of NTDs) there is a caudal defect in which the spinal cord is dysplastic and the overlying spinal column is absent. It is not usually fatal but can cause paraplegia, with paralysis of the lower extremities and impaired bladder and bowel function (Pitkin, 2007). Infants born with NTDs have increased risk of mortality within the first year of life,

and survivors face life-long morbidities including neurologic, cognitive, urologic, and gastrointestinal complications (Marean et al., 2011). NTDs are devastating conditions as most of the lesions are always associated with neurological deficits producing varying degree of limb paresis/paralysis, bladder and anorectal incontinence (Digra, 2004).

NTDs are multifactorial as are believed to reflect a combination of genetic predisposition and environmental influences (Penchaszadeh, 2002). It may be the result of genetic abnormalities, the intrauterine environment, morphogenesis errors, or a chromosomal abnormality. The wide geographic variations in incidence suggest the prime role of importance of intra uterine environmental factors (Penchaszadeh, 2002). Neural tube defects indeed are particularly prevalent in China (Xiao et al., 1990), Mexico (Mutchinik, 1988), Central America (Saborio, 1992) and Chile (Nazer et al., 2001). Among intrauterine environmental factors, nutrition plays the most critical role in fetal growth and development (Belkacemi et al., 2010). Maternal under nutrition during gestation reduces placental and fetal growth of both domestic animals and humans (Barker & Clark, 1997; Redmer et al., 2004; Wu et al., 2006). Substantial evidence suggests that fetal growth is most susceptible to maternal dietary deficiencies of nutrients (protein and micronutrients) during the peri-implantation period and the period of rapid placental development (Sugden & Holness, 2002; Waterland & Jirtle, 2004; Wu et al., 2004). During pregnancy, when the woman's nutritional intake also provide for the growing foetus, a woman's requirement for numerous micronutrients mainly folate, Vitamin A, C, D, K, B12 is on the rise. Among these, folate is a B vitamin which women require in increased amounts during pregnancy - to assist with cell division in the baby. Intrauterine folic acid deficiency is a well known factor predisposing to neural tube defects and possibly other congenital anomalies (Czeizel & Dudás, 1992).

3. Folate versus folic acid

The generic term "folate" represents the complete group of all form of folate, a water-soluble B vitamin, including synthesized fully-oxidized “folic acid” commonly used for food fortification and nutritional supplements.and the polylglutamates naturally present in foods (Iyer & Tomar, 2009). Folic acid or pteroyl glutamic acid (PGA) is comprised of *p*-aminobenzoic acid (PABA) linked at one end to a pteridine ring and at the other end to L-glutamic acid (Fig. 1a). The naturally occurring forms of folate differ in the extent of the reduction state of the pteroyl group, the nature of the substituents on the pteridine ring and the number of glutamyl residues attached to the pteroyl group (Fig. 1b).

Good dietary sources of folate include breakfast cereals; other cereals or cereal based foods (e.g. bread); yeast extract; beans and legumes etc. Natural food folates or pteroylpolyglutamates are conjugated to a polyglutamyl chain containing different numbers of glutamic acids which is removed in the brush border of the mucosal cells by the enzyme folate conjugase (Scott & Weir, 1994). Subsequently the polylglutamates are hydrolyzed to monoglutamate prior to absorption in the small intestine. The primary form of folate entering human circulation from the intestinal cells is 5-methyltetrahydrofolate monoglutamate. If enough folic acid is given orally, unaltered folic acid appears in the circulation (Kelly et al., 1997), is taken up by cells, and is reduced by dihydrofolate reductase to tetrahydrofolate.

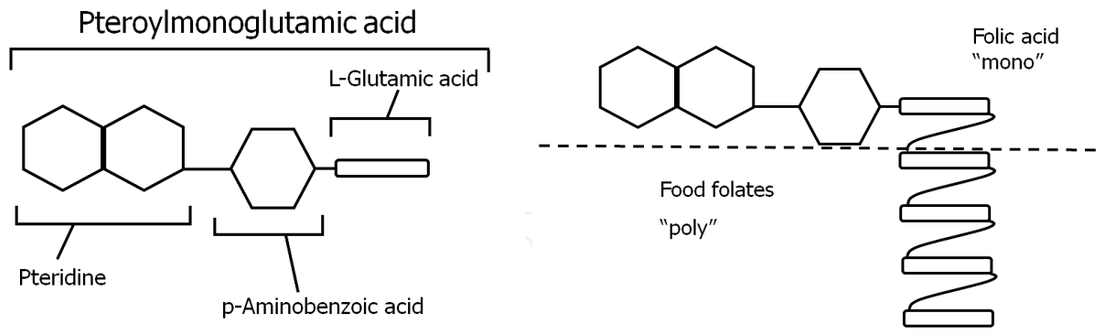


Fig. 1. Structure of a) folic acid (pteroyl-L-glutamic acid), and b) native food folates, e.g. reduced, one-carbonsubstituted forms of polyglutamates.

The bio-availability of natural folates is affected by the removal of the polyglutamate chain by the intestinal conjugase and therefore the monoglutamate forms, including folic acid, are easily transported across small intestine (Shils et al., 1994; Hendler & Rorvik, 2001). The absorption efficiency of natural folates is approximately half from that of synthetic folic acid. As folate requires hydrolysis to monomeric forms before it can be used thus relative bioavailability of dietary folates is estimated to be only 50% compared with synthetic folic acid (Sauberlich et al., 1987; Forssen et al., 2000; Fitzpatrick, 2003; Iyer & Tomar, 2009).

3.1 Folate deficiency

Folate is an essential nutrient component of normal human diet involved in numerous metabolic reactions as DNA and RNA biosynthesis and amino acid inter-conversions (Iyer & Tomar, 2009). Functional folates have one-carbon groups derived from several metabolic precursors (e.g., serine, *N*-formino-L-glutamate, folate, etc.). The DNA and methylation cycles both regenerate tetrahydrofolate (Fig.2.).

However, there is a considerable amount of catabolism of folate and hence there is always a need to replenish the body's folate content by uptake from the diet. If there is inadequate dietary folate, the activity of both DNA and methylation cycles will be reduced and thereby affects cell division and amino acid interconversion. Folate deficiency has been implicated in a wide variety of disorders from Alzheimer's to coronary heart diseases, neural tube defects, anemia, increased risk of breast and colorectal cancer (Verhoef et al., 1998; Verhaar et al., 2002; Le Blancet al., 2007; Tomar & Iyer, 2011). Although folate is found in a wide variety of foods, it is present in a relatively low density (Chanarin, 1979).

Though a normal human diet has sufficient amount of folate, still folate deficiencies occur frequently, even in well-developed countries (Konings et al., 2001; O'Brien et al., 2001). As activity of natural folates tend to rapidly diminish over periods of days or weeks hence a significant loss of biochemical activity is liable to occur during harvesting, storage, distribution, and cooking. Moreover owing to inability of the mammalian cells to synthesize this vital biomolecule; an external supply is essential to prevent nutritional deficiency.

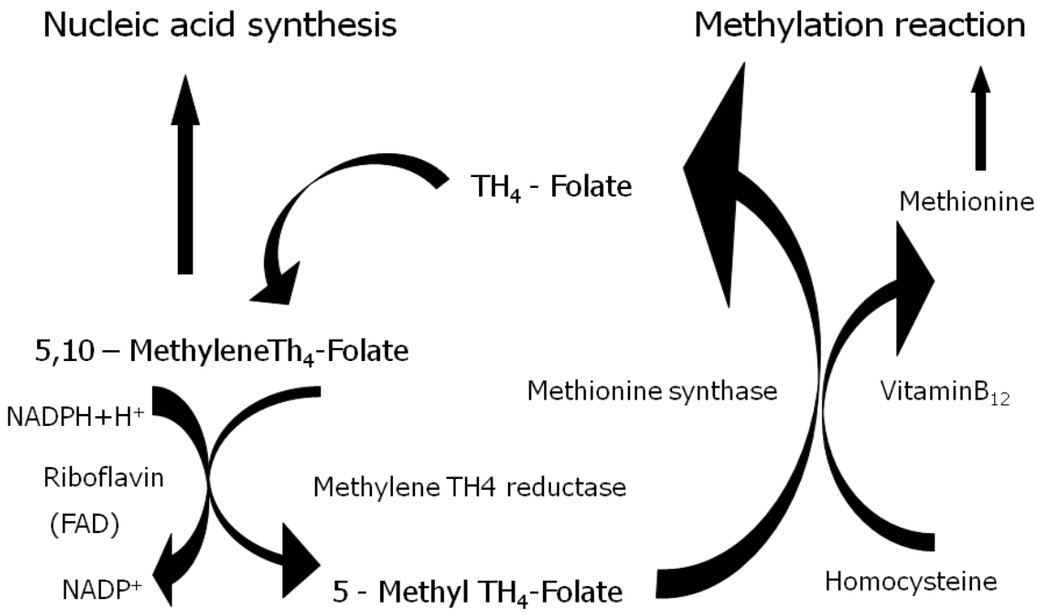


Fig. 2. Role of folate in metabolism

3.2 Role of folic acid in NTD pathophysiology

Besides the common reasons of malnutrition, malabsorption and low intake, increased requirement is also one of the important cause of folate deficiency. As folate plays an essential role in cellular division, pregnancy doubles the need of dietary folates (Forssen et al., 2000; Patterson et al., 2008). Pregnancy is associated with a marked acceleration in 1-carbon transfer reactions, including those required for nucleotide synthesis and thus cell division, which is the basis for the substantial increase in folate requirements during pregnancy. Low maternal folate status has been associated with premature birth, low birth-weight and increased risk of NTDs in the offspring (Iyer & Tomar, 2009; Kim et al., 2011).

The possibility that folic acid played a vital in NTD role was first reported by Hibbard (1964) and further scientists in 1976, observed that women who gave birth to NTD babies had low serum rates for folates and low vitamin levels in their red cells. Later Professor Smithhells in 1976, showed that an additional intake of 0.4 mgs of folic acid prior and at the initiation of a pregnancy caused significant drop in NTD rates. Several clinical trials subsequently demonstrated that the risk of recurrence and first occurrence of these abnormalities were declined by periconceptional folic acid supplementation prior to and during pregnancy (MRC vitamin study research group, 1991; Czeizel & Dudas, 1992; Werler et al., 1993; Czeizel et al., 1993). Several other studies have also shown that further related congenital anomalies can be prevented by folic acid intake (Czeizel 1993, Antony & Hansen, 2000). Besides, a good number of studies indicate that folic acid could potentially reduce the risk of miscarriage (George et al., 2002). Also reports are there which shows that folic acid supplementation before conception may potentially reduce the frequency of Down's syndrome (James et al., 1999; Barkai et al., 2003; Patterson, 2008).

Despite the importance of folate in NTD prevention, the mechanism by which folic acid exerts its preventive effect is unknown. Moreover folic acid also induces epigenetic changes during development and can conceivably affect the epigenetic regulation of gene expression (Lillycrop et al., 2005; Mathers et al., 2010; Li et al., 2011). Thus, the identification of genetic risk factors for human NTDs is complicated by the multiplicity of genes participating in neurulation, and the importance of gene–environment interactions (Greene et al., 2009a, b). Research on NTD pathophysiology suggests several gene defects affecting enzymes and proteins involved in transport and metabolism of folate have been associated with NTDs (Carroll et al., 2009). Among them methionine synthase activity is one of the important factors involved. This enzyme transforms homocysteine into methionine for which folic acid acts as methyl group donor. Besides folate and vitamin B12 concentrations being the independent risk factors for NTDs, homocysteine concentrations also mildly get increased in maternal blood and amniotic fluid of NTD pregnancies (Kirke et al., 1993) which signifies the potential role of disregulated methionine synthase in NTDs development. Moreover, genetic association between the methionine synthase gene and NTDs in affected families remains ambiguous (De Marco et al., 2002; Pulikkunnel & Thomas, 2005; Doudney et al., 2009). Though the role of enzyme 5, 10-methylene tetrahydrofolate reductase (MTHFR) in the NTD etiology is well documented (Van der Put et al., 1995), but is still debatable (de Franchis et al., 1995; Koch et al., 1998). Thus, folic acid deficiency or an enzyme anomaly prevents the closure of the neural tube.

In humans, most genetic causes of NTDs remain unknown. Fleming & Copp, (1998) identified a mouse model of folate- preventable NTD using deoxyuridine suppression test so as to detect disturbance of folate metabolism in homozygous *splotch* (*Pax3*) mouse embryos developing NTDs *in vitro.* They observed that as folic acid and thymidine can prevent NTDs in *splotch* embryos, having abnormal pyrimidine biosynthesis which mainly leads to NTD,and hence suggested that thymidine therapy could serve as an adjunct to folic acid supplementation to prevent human NTDs. Till date mouse models of NTDs are beginning to provide insight into the genetic causes and developmental origins of NTDs in humans (Greene et al., 2009b) and over 240 genes are identified to be important in neural tube closure in mice (Harris & Juriloff, 2010). Moreover, mouse NTD models have the potential to aid in understanding the genetics underlying folic acid responsiveness or non-responsiveness in NTD prevention (Harris, 2009). Recently Marean et al., 2011 suggested that the response to folic acid supplementation may be more complex. They report that depending on the gene mutation, folic acid supplementation may adversely influence embryonic development and neural tube closure and thus the genetics of an individual may determine whether FA provides a beneficial outcome.

Therefore several reports support the hypothesis that folic acid likely acts through diverse mechanisms to influence neural tube closure (Li et al., 2011; Marean et al., 2011) so in future it will be of interest to determine the mechanistic basis of the specific gene-environment interactions that together influence neural tube closure.

3.3 Biomarkers to estimate folate status and intake

Measuring folate intake at the population level would be sufficient to predict NTD occurrence as insufficient folate intake being one of the major cause of NTD. The Food and Agriculture Organization of the United Nations and World Health Organization

(FAO/WHO) Expert Consultation report (FAO/WHO, 1988) suggested that adequate folate status is reflected in a red cell folate level of greater than 150 mg/L. RBC-folate is a effective biomarker that may reflect probable conditions that exist in other cells in the organism and therefore explain the occurrence of NTD (Dary, 2009). Several irrefutable evidences maintain that lower red cell folate, earlier considered as an adequate or normal range, is associated with an increased risk of spina bifida and other NTDs (Kirke et al., 1993). Red cell folate levels higher than 150 μg/L, which are adequate to prevent anaemia are associated with increase risk of NTDs (Daly, 1995; 1997) and colorectal cancer (Mason, 1995; Kim, 2004). Though RBC-folate is a good indicator of the long term folate status, but is influenced by an individual's genetic composition, and the availability of sufficient amounts of iron, zinc, protein, vitamins, and other nutrients which influence the synthesis of RBC and reactions invoving folate (Bailey & Gregory, 1999; Mc Nulty & Scott , 2008).

Apart from red cell folate which is an important index of folate status (Sauberlich, 1995) plasma folate and indicators of haematologic status such as raised mean corpuscular volume, and hypersegmentation of neutrophils remains important indicators of reduced folate status (Lindenbaum et al., 1990). To establish folate intake serum/plasma folate level is a more robust biomarker than RBC-folate, for it reflects the recent folate intake and it is less affected by confounding factors. Though it may vary on a daily basis at individual level, it is satisfactory enough to support population-based folic acid interventions programs. Besides this, the biomarker plasma homo-cysteine is also a very sensitive indicator of folate status and must be added as folate adequacy indicator. But this can be done on an individual basis only after nullifying the chance of a genetic mutation or an inadequate supply of vitamin B_6 or vitamin B_{12}. In one-carbon metabolism, many pathologies (nutritional and genetic) beyond folate intake can cause abnormalities. Therefore, serum folate should be used along with other biomarkers, such as RBC-folate, blood homocysteine, and others, to identify pathological causes and possible treatments for normalizing abnormal pathways.

4. Recommended daily allowance of folate

The US National Academy of Sciences in a series of reports (IOM, 1998) thoroughly evaluated the evidences of folate intake, status in context to health for all age groups and the extra requirements during pregnancy and lactation. Based on the FAO/WHO recommended nutrient intake (RNI), and members of this FAO/WHO expert group the values as recommended dietary allowances (RDAs) were published by the US National Academy of Sciences as the best estimates of folate requirements for healthy individuals and populations. The Recommended Daily Allowance (RDA) for folate recommendations in most countries are therefore set to 400 micrograms (mcg) per day for all adults (Yates et al., 1998). The RDA for folate is expressed in Dietary Folate Equivalents (DFE), which accounts for the difference in absorption between dietary folate and synthetic folic acid, which is more bioavailable (in a form that is easily used by the body).

A woman's folate requirement escalates by 50% during pregnancy with highest in the first trimester of pregnancy. It is important to note that this requirement does not include the additional folate necessary to prevent neural tube defects, as folate intake prior to becoming pregnant largely determines the risk of neural tube defect. Neural tube fully developed between 22 and 28 days after conception (3-4 weeks), during which many women are not

even aware about their pregnancy. It is therefore also important for women of childbearing age who are planning a pregnancy or might become pregnant to ensure they consume the recommended quantities of folate for at least one month pre-pregnancy. Researchers have found that 50-70% of NTDs can be prevented if women consume adequate amounts of folate before and in the first trimester of pregnancy. Based on the evidence that multivitamins and foods containing folic acid besides protecting against NTD, also prevent other types of birth defects, including cleft palate and cleft lip and some cardiovascular malformations, the Food and Nutrition Board issued new dietary recommendations for folic acid, recognizing the need for women of childbearing age to get supplemental folic acid, over and above the amounts that are naturally present in foods (Food and Nutrition Board, 1998).

The US Centers for Disease Control (CDC) recommends all women of childbearing age eat a diet high in folic acid or take a multivitamin with 0.4mg of folic acid each day, especially one month prior to conception through the first three months of pregnancy. However, women who have had a previous NTD pregnancy are recommended to take an even higher dosage of folic acid from 0.4mg to 4.0mg, prior to planning a pregnancy. In Canada, the recommendation is to begin three months before pregnancy. The RDA of folate for all pregnant women is 600µg, compared to 400µg for non-pregnant women. As folic acid is also important for lactating women so to fulfill the demands of breastfeeding, the RDA for lactating women in the United States is 500 μg dietary folate equivalents (DFE) day.

4.1 Folic acid supplementation programs

As many pregnancies are unplanned, and neural tube develops in the prelims of pregnancy so NTD prevention is best achieved by adequate daily folic acid intake throughout the reproductive years. Though possible for women to increase their consumption of dietary folates by careful selection of foods, but food folates are about half as bioavailable as synthetic folic acid (Gregory, 1997). The United States Public Health Service recommended that women capable of becoming pregnant should consume 400 ug of folic acid daily (CDC, 1992; Cornel & Erickson, 1997; Penchaszadeh, 2002). The only viable and sustainable preventive strategy for this is fortification of food with folic acid. Fortification of staple food products with synthetic folic acid is an efficient and economical approach to increasing overall folic acid intake and fulfilling RDA.

The intricacy with any fortification programme is that people having low intakes of the fortified food must consume sufficient amount to benefit, whereas those consuming high amounts must be prevented from receiving a potentially harmful dose. An assets of information in folate biochemistry, molecular biology, human and population genetics has been build up that can be used to direct public health decisions on folate interventions. Keeping this in mind, the Food and Drug Administration (1996) mandated the fortification of enriched cereal grain flours with 140 µg synthetic folic acid per 100 grams of grain. Later in 1999 the Ministry of Health of Chile, South America, issued a regulation of folic acid supplementation to the wheat flour premix at a concentration of 2.2 mg/kg of flour (Nazer et al., 2001). This policy was encouraged and implemented by the Pan American Health Organization, the March of Dimes and the CDC with an additional monitoring of its effect on the NTD prevalence (Nazer et al., 2001). As many as 40 countries including United States, Canada, and Israel, have also implemented mass-fortification programs of food supply (usually flour) fortification with folic acid so as to bring folic acid to women of childbearing

age so as to prevent folic acid-related birth defects. Such studies showed that fortification programs led to 31% and 16%reduction in prevalence of spina bifida and anencephaly. Some of these programs involve education of mothers-to-be about the importance of taking folic acid supplements prior to conception and during pregnancy (World Health Organization and Food and Agriculture Organization, 2006).

Efficacious amounts of folic acid can also be supplied by both daily (≈100 mg) and weekly (≈400 mg) folic acid supplementation (Adank et al., 2003; Norsworthy et al., 2004; Hao et al., 2008). However, the population dosage should be adjusted based on carefully monitoring of serum folate as biomarker so that the folate and folic acid intakes of most individuals remain within the safety level. Although this is a step ahead to prevent folic acid deficiency but, the actual amount added may not be sufficient to protect against all folic acid-preventable NTDs. Though Vitamin B12 deficiency is rare in women and children, there is apprehension that folic acid dosages exceeding 1000ug (1.0 mg) per day may hamper the Vit B12 diagnosis. Mills et al., (2003) however, indicates that fortification does not lead to a major increase in masking of vitamin B12 deficiency. Hence, the recommended amounts of folic acid obtained from folic acid supplements and food fortification are unlikely to exceed the 1000 ug per day.

5. Controversies related to folate versus folic acid fortification

Given the available evidence, the fortification of foods with folic acid is justifiable. It is an effective and inexpensive way to ensure adequate folate levels in all prospective mothers and maximizes the effect of folic acid in preventing NTDs (Kadir & Economides, 2002). Irrespective of folic acid supplementation's benefits for mothers-to-be, there is debate about the necessity of putting folic acid in the food supply. There has been discussion about the long-term effects of food fortification, as well as what effects folic acid may have on the general population, who would also be consuming the fortified foods.

Dietary folate polyglutamates, is enzymatically deconjugated at the mucosal epithelial cell brush border by conjugase to the corresponding monoglutamate forms before absorption (Chandler et al., 1986; Krishnaswamy & Madhavan, 2001). Folic acid and reduced monoglutamyl folates are absorbed mainly in the proximal small intestine (jejunum) by a saturable, carrier-mediated, pH and energy-dependent transport mechanism which, shows a similar affinity for both oxidized (e.g. folic acid) and reduced folate forms (Strum, 1976; Mason, 1990). On passage through the intestinal wall, physiological doses of folic acid undergo biotransformation in the absorptive cells of the upper small intestine to 5-methyltetrahydrofolic acid, the naturally circulating form of folate. Contrary to this, folic acid, the synthetic form which is used in nutritional supplements and fortified foods is very efficiently absorbed by the body (Fig.3.). However, some studies have indicated that oral dosage of folic acid in high doses may overwhelm this conversion pathway, leading to the direct appearance of measurable levels of untransformed folic acid in the systemic circulation of man (Kelly et al., 1997; Wright et al., 2007). This indicating a saturation point, is an evidence that intestinal conversion is not a prerequisite for transport, and is arguably indicative of the dividing line between physiological and nonphysiological oral doses of folate.

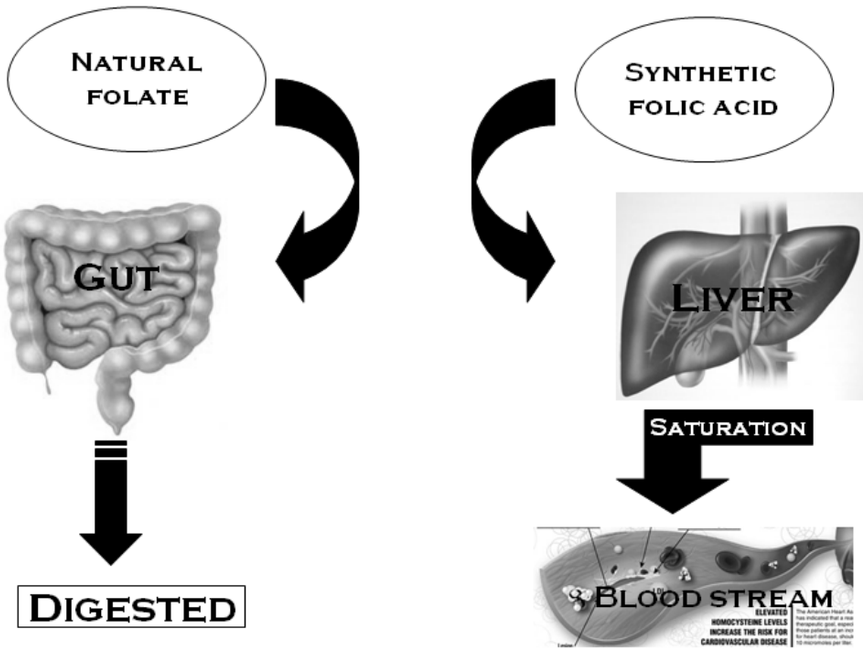


Fig. 3. Fate of natural folate and synthetic folic acid in the gut

Orhvik et al., (2010) determined folate bioavailability after ingestion of breads or a breakfast meal fortified with either folate or folic acid by using a stable-isotope area under the curve and ileostomy model and observed that there is a difference in plasma absorption kinetics for reduced folates and synthetic folic acid administered with the test foods. Also stomal folate contents indicated almost complete bioavailability of labeled folate from the breads or breakfast meal. Therefore, based on a re-appraisal of historical literature it is now hypothesised that folic acid metabolism in man primarily occurs in the liver, on contrary to absorptive cells of the upper small intestine where folate gets metabolized. Therefore due to human liver's low capacity for reduction it eventually gets saturated, resulting in significant and potentially deleterious unmetabolised folic acid entering the systemic circulation. In consequence of limited data available for folate bioavailability of vegetables, fruits, cereal products, and fortified foods, and difficultly in evaluation of bioavailability of food folate Orhvik et al., 2011 recommended to revisie the classical approach of using folic acid as a reference dose for estimating the plasma kinetics and relative bioavailability of food folate. Although a criterion for determining an excessive folate status has not yet been specified, a level above 45 nmol/L has been considered suitable as presence of free-circulating folic acid indicates that the organism's capacity to transform folic acid into folate derivatives has been overwhelmed (Pfeiffer et al., 1997; 2005; Dary , 2009).

A number of studies have shown that this high circulating synthetic folic acid supplements provoke a number of health complaints such as the masking of the early hematological manifestations of vitamin B12 deficiency, leukemia, arthritis, bowel cancer and ectopic pregnancies (Lucock and Yates, 2005; Sweeney et al., 2007; Wright et al., 2007). The other potential risks are interference with folate antagonistic drugs, zinc malabsorption and hypersensitivity reactions (Kadir & Economides, 2002). Though the risk of toxicity and harmful side effects from too much folic acid is low, since it is water soluble and human

body excretes any excess that is not absorbed. The tolerable upper intake level for folic acid is 1,000 mcg, reports the Office of Dietary Supplements. Consuming more than this amount may cause adverse effects and mask the absorption of other nutrients.

Therefore before mandatory folic acid fortification is implemented, it is essential that a thorough assessment of all the potential concerns should be addressed precisely to ascertain a true picture of risk/benefit of fortification. To evaluate all the potential benefits and risks and optimal intakes of folic acid for all segments of the population, the physiologic and safety ramifications of lifetime exposure to circulating folic acid need to be elucidated (Kalmbach et al., 2008). Also a minimum efficacious level must be selected that prevent the consequences of folate deficiency yet minimizes the adverse effects associated with excesses.

6. Biofortification – A novel natural folate fortification approach

Since the role of the diet and fortified foods is still dubious, determination of the relative efficacy of food folate, folic acid added to foods, and supplemental folic acid alone is one of important question which needs to be answered. As highlighted by FDA, 1996, high intakes of chemically synthesized folic acid, (tolerable upper intake level, 1000 μg d^{-1}), can cause several adverse health effects as masking of macrocytic anemia, leukemia, arthritis, bowel cancer. For these reasons, many researchers have been critically evaluating the dietary sources of folates and looking for novel methods to increase concentrations of naturally occurring folate variants in foods instead of supplements and tablets. The enhancement of folate contents in staple food through genetic modification, so-called biofortification, can offer an alternative or at least complement the current methods to enhance intakes of natural food folates (Finglas, 2006). Biofortified crops offer potential advantages over supplementation or fortification strategies as seeds can be re-sown every year from the saved harvest. Recent progress in plant genomics of the model plants (The Arabidopsis Genome Initiative, 2000; Yu et al., 2002), in conjugation with available knowledge of folate biosynthesis biochemistry (Scott et al., 2000), has made feasible the folate enhancement by biofortification.

Product	**Folate (μg l^{-1})**
Milk	40 ± 10
Buttermilk	90 ± 20
Dahi	60± 20
Yogurt	80 ± 20
Acidophilus milk	50 ± 10
Bifidus milk	75 ± 15

Table 1. Folate concentrations in dairy products and its contribution to the RDI

Besides genetic manipulation, the vegetables (broccoli, cauliflower), legumes (beans, nuts, peas, etc.) leafy greens (such as spinach), citrus fruits, liver (Eitenmiller & Landen, 1999; Forssen et al., 2000; Iyer & Tomar, 2009) milk and fermented dairy products represent an important dietary sources of folates (Lin & Young, 2000; Iyer et al., 2009). However, many dairy products are processed using microbial fermentations in which folate can be synthesized (Table.1), significantly increasing folate concentrations in the final product (Lin & Young, 2000; Iyer & Tomar, 2009). Therefore in some cases, fermented milk products are

reported to contain such higher amounts of folate, that with an average serving of the product, the RDA for the vitamin would be met, or exceeded (Sybesma *et al*., 2003).

This high level is the result of the production of additional folates by bacteria such as as *Lactococcus lactis, S. thermophilus, Leuconostoc species, Bifidobacterium longum* and some strains of *Propionibacteria* (Lin & Young, 2000; Hugenholtz et al., 2002; Crittenden et al., 2003; Papapstoyiannidis et al., 2006; Tomar & Iyer, 2011; Iyer et al., 2011). The ability to produce folate can differ (Table.2) remarkably between different lactic acid bacteria.

Microbial Species	**Total (µg)**
Lc. Lactis subsp cremoris	92-116
Lc. Lactis subsp lactis	57-291
L.plantarum	45
L.acidophilus	1
L.casei subsp *rhamnosus*	-63
L.delbrueckii subsp. *bulgaricus*	54
P.acidipropionici	36
P.frendenreichii ssp. *shermanii*	17-78
B. adolescents	70-110
S. thermophilus	29-202
Leuconostoc lactis	45
Sacchromyces spp.	40

Table 2. Folate produced by different microorganisms

Keeping in view the potential risks of fortification with folic acid, fermented milks containing elevated levels of natural form folates seems to be more rationale for fortification purposes (Scott, 1999; Iyer & Tomar, 2011). Fermented milks can be a potential food matrix among dairy products for folate fortification because folate binding proteins of milk improve folate stability and the bioavailability of both 5-methyltetrahydrofolate and folic acid may be enhanced (Jones & Nixon, 2002; Aryana, 2003; Verwei et al., 2003). Hence, is an interesting strategy to increase "natural" folate levels in foods. Hence, an interesting strategy to increase "natural" folate levels in foods is by fermentation fortification by by the judicious selection and exploitation of high folate producing microorganisms. Therefore the application of high folate producing microorganisms could lead to the production of fermented dairy and other food products with increased levels of natural folate and can be a part of strategy for the economic development of novel functional foods with increased nutritional value.

7. Conclusion

Over the past half century one of the most exciting scientific developments authenticated by a chain of clinical research studies is the finding that folic acid plays a critical role in protecting against NTD. This article summarizes in brief the more important topics which have been either explored or need to be explored, which have led to the current situation in which all women capable of becoming pregnant are urged to ingest folic acid regularly. Though folate is present in various foods constituting our ordinary diet, yet is insufficient to meet RDA, which makes us vulnerable to folate deficiency. This problem can be addressed

either by fortifying foods with folic acids or by use of folate rich foods and fermentation fortification by employing folate synthesizing food grade bacteria to increase the *in situ* folate levels in fermented foods (LeBlanc et al. 2007; Tomar and Iyer, 2011). In consequence to the health benefits associated with increased folate intakes many countries now possess mandatory folate enrichment programs. Although some controversy remains about the adequacy of fortification levels, the adverse effects of synthetic folic acid in contrast to natural food folate and genetic of folate in NTD. Nonetheless, several gaps in knowledge still need to be filled. The most important research need relates to our incomplete understanding of the mechanism and genetics by which the NTD defect occurs and how folate influence it to expand the scientific discussion of best health policy practices. The need for mechanistic knowledge is more so as to identify high-risk subjects and develop more effective interventions with an aim to obtain better results with folic acid supplementation .Besides this there is an urgent need to accurately delineate the dose-response relations of folate and NTD prevention so as to solve the current debate about appropriate levels of food fortification. Development of biofortified staple and vegetable crops with enhanced folate contents and their implementation is urgently required (Finglas, 2006).

Research should also be accompanied by economic feasibility studies that compare biofortification to conventional supplementation and fortification strategies. Simultaneously farmer and consumer acceptance studies should also go hand in hand in order to determine the acceptability of transgenic cereals with enhanced folate levels. Further, more strict regulatory measures for monitoring folic acid content in industrially processed foods and other products should be followed efficiently to complement mass-fortification programs. Apart from all this folic acid education campaigns need to be orchestrated to increase the knowledge about the benefits and sources of folic acid, and especially the correct, periconceptional timing of folic acid intake to reduce the overall incidence of NTDs (Rofai et al., 2011). For successful health campaign research besides identifying and reaching target groups, there is an immense need of awareness programs within high school and university educational system.

8. References

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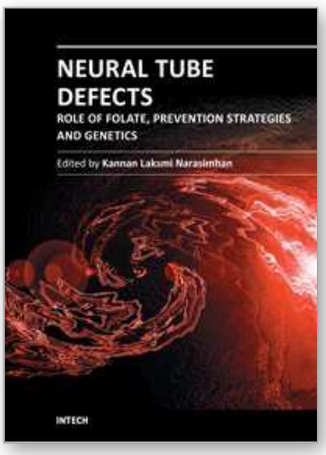
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