CHAPTER FIVE

Recent Developments in Folate Nutrition

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Abstract

The term folate (vitamin B9) refers to a group of water-soluble compounds that are nutritionally essential for the support of optimal human health and development. Folates participate in numerous one-carbon transfer reactions, including the methylation of important biomolecules (lipids, amino acids, DNA). A deficiency of folate leads to pathological outcomes including anemia and impairments in reproductive health and fetal development. Due to the linkage of impaired folate status with an increased prevalence of neural tube defects (NTDs) in babies, several jurisdictions required the fortification of the food supply with folic acid, a synthetic and stable form of folate. Data from the postfortification era have provided strong evidence for the reduction of NTDs due to folic acid fortification. However, concern is now growing with respect to the amount of synthetic folic acid within the human food supply. Excess folic acid intake has been linked to a masking of vitamin B12 deficiency, and concerns regarding the promotion of folate-sensitive cancers, including colorectal cancer. New strategies to ensure the supply of optimal folate to at-risk populations may be needed, including the use of biofortification approaches, in order to address recent concerns.

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1. INTRODUCTION

Folate (vitamin B9) represents the collective term for a class of compounds (vitamers) belonging to the water-soluble family of vitamins. Collectively, the folate vitamers play important roles in one-carbon metabolism, including their involvement in the biosynthetic pathways for key amino acids and nucleic acids (Institute of Medicine, 1998). As such, folate is a key factor in cell replication and intermediary metabolism. A deficiency of folate has been linked to a variety of disorders (Iyer & Tomar, 2009), including anemia and neural tube defects (NTDs). While animal cells can interconvert the various vitamin forms, they do not possess the ability to synthesize the foundational pteroylglutamic acid structure, and thus rely on exogenous sources of this important nutrient (Molloy, 2005). Exogenous supply can come in the form of naturally occurring folates in the food supply and via folate synthesized by commensal microorganisms within the large bowel. Additionally, synthetic crystalline folic acid is routinely used to fortify foods and as a constituent of dietary supplements. While supplemental folic acid has proven effective in addressing critical public health issues, including the reduction of the incidence of NTDs, there is growing evidence that the human food supply is now overfortified with folic acid, and this might pose health risks, including the increased risk of developing colorectal cancer and the masking of vitamin B12 deficiency (Kim, 2004). The risk appears linked to crystalline folic acid, and not the naturally occurring forms of folate. As such, there is interest in positioning alternative sources of folate for use in supplements and to fortify the food supply. This chapter will further discuss recent advances in our understanding of folate nutrition, particularly with respect to addressing alternative approaches to ensure adequate folate intakes in target populations.

2. FOLATE AND FOLIC ACID

2.1 Terminology and Chemical Structures

Folate, also known as folacin or vitamin B9, refers to a family of chemically and functionally related compounds that exhibit a common vitamin activity based on the parent structure of folic acid (Selhub & Rosenberg, 1996). The synthetic folic acid (pteroylglutamate) is the most oxidized and stable form of folate. Folic acid (molecular weight = 441.4 (g/mol)) is

odorless, slightly soluble in cold water, with more solubility in aqueous alkali, acetic acid, phenol, pyridine, and other basic solvents. Folic acid is fully oxidized and, in neutral solution, has a maximum absorption at 298 nm. Synthetic folic acid lacks coenzyme activity and must be reduced to the metabolically active tetrahydrofolate (THF) form within the cell. Due to its chemical stability and greater commercial availability compared to natural form of folate, folic acid is the most commonly used form in supplements and food fortification. Both natural folate and synthetic folic acid after intakes are converted to 5-methyltetrahydrofolate (5-MTHF), and this is the predominant folate form usually found in blood plasma (Wien et al., 2012).

The basic structure of folate comprises a pteridine ring attached to *p*-aminobenzoic acid through a methylene group, an L-glutamic acid residue is linked to *p*-aminobenzoic acid (Fig. 1). The terms folate and folic acid are often used interchangeably for this vitamin. The pteridine ring of folic acid is fully oxidized, while natural folates occur as dihydro- or tetrahydrofolates (H_4folate) with different one-carbon substituents linked at the N5- and/or N10-position (Fig. 2; Lavoisier, 2008).

The folates in food and feedstuffs are typically in a reduced form as polyglutamyl derivatives of tetrahydrofolic acid. The polyglutamated form of 5-MTHF is recognized as the predominant form of dietary folate and is naturally found in fresh food sources (Lucock, 2000). The 5-MTHF vitamer has the chemical formula $C_{20}H_{25}N_7O_6$ (molecular weight of 459.5 g/mol) and possesses intermediate oxidative stability relative to folic acid. The

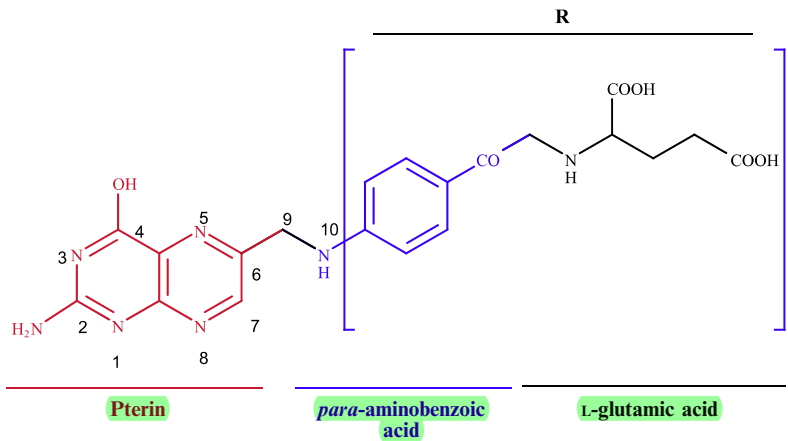

Fig. 1 Chemical structure of crystalline folic acid.

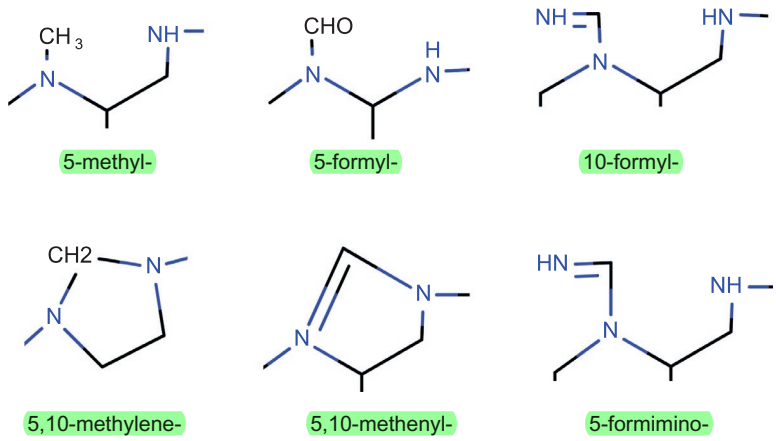

Fig. 2 One-carbon moieties linked at either the N5- and/or N10-position of the folate structure.

compound can be isolated as a white powder; however, in solution it requires ascorbate or another reducing substance to achieve medium-term stability. At neutral pH, it has an absorption maxima of 290 (Scott & Weir, 1993). A calcium salt of L-5-methyltetrahydrofolic acid ($C_{20}H_{23}CaN_7O_6$; MW = 497.5 g/mol) has been synthesized (Ginting & Arcot, 2004) and shown to possess comparative physiological activity, bioavailability, and absorption at equimolar doses to the naturally occurring form (Pietrzik, Bailey, & Shane, 2010).

2.2 Historical Perspective

The natural form of folate was first discovered by Lucy Wills over 80 years ago as a component of yeast with documented abilities to both prevent and cure macrocytic anemia. The structure of polyglutamate folate was determined in the mid-1940s. The chronology of folate discovery is summarized in Table 1. Later in 1943, the monoglutamate form of folate was synthesized in pure crystalline form, and this discovery proved that folic acid is composed of a pteridine ring, *p*-aminobenzoic acid, and glutamic acid. Afterward, it became evident that natural folates usually differed from pteroylglutamic acid. Today, folic acid refers to the fully oxidized chemical compound which does not exist in natural foods. The term "folate" is designated to the large group of compounds having the same vitamin activity and includes natural folates and folic acid (Hoffbrand & Weir, 2001).

Table 1 The Chronology of Folic Acid Discovery From Ulrich and Potter (2006)

1930	Wills and Mehta	Yeast extract prevented the dietary anemia in rats
1931	Wills	Yeast or marmite prevents macrocytic anemia of pregnancy
1934	Vaughan and Turnbull	Marmite corrects anemia of coeliac disease
1938	Wills and Evans	Purified liver extracts do not correct nutritional, pregnancy, or macrocytic anemia
1940	Snell and Peterson	Norit eluate factor—factor absorbed from yeast or liver is growth factor for *Lactobacillus casei*
1941	Mitchell, Snell, and Williams	The term folic acid coined and shown to be a growth factor for *Streptococcus lactis R* (*Streptococcus faecalis*)
1943; 1946	Fullerton; Watson and Castle	Idiopathic steatorrhoea megaloblastic anemia responds to crude liver extracts or yeast extract
1943	Wright and Welch	Enzyme hydrolyzing folate polyglutamates to monoglutamates folate conjugase
1944	Binkley et al.	Yeast extracts effective as a source of vitamin BC only 2%–5% being active for *L. casei*. Required enzymatic digestion to balance activity
1945	Angier et al.	Synthesis of folic acid and using the term pteroylglutamic acid
1945	Day et al.	Purified *L. casei* factor is vitamin M
1946	Pfiffner, Calkins, Bloom, and O'Dell	Naturally occurring folate in liver is a heptaglutamate

2.3 Functional Bioefficacy

The functional bioefficacy of a nutrient refers to the proportion of it that, once ingested, possesses the ability to perform specific metabolic function (Brouwer, van Dusseldorp, West, & Steegers-Theunissen, 2001). Since plasma total homocysteine is a functional index of folate status, by nature of the role that folate plays in sulfur amino acid metabolism (Fig. 3), changes in plasma total homocysteine concentration in response to a given intake of folate or folic acid can be used as a measure of functional bioefficacy. Factors influencing bioavailability and bioefficacy of folate include: (a) species of folate, (b) amount of folate and folic acid consumed, (c) food matrix effects

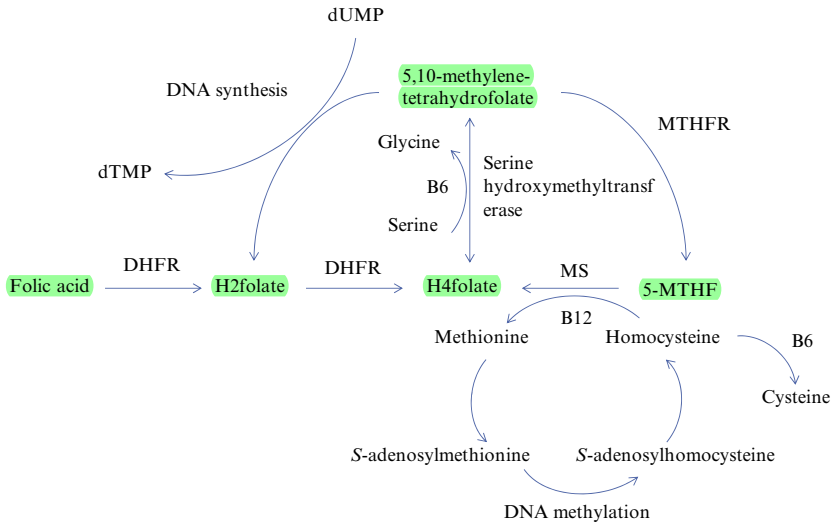

Fig. 3 Schematic presentation of the main metabolic pathways of folate.

(d) overall nutrient status of the host (e) genetic factors, and (f) host-related factors (Gregory, 2001).

Folates and their derivatives occur in nature in forms ranging from mono- to polyglutamates. Synthetic folic acid exists in the monoglutamate form and it is fully oxidized at the N5- and N10-positions. The polyglutamated forms of natural folates, a form needed for cellular folate retention, must be hydrolyzed to monoglutamate forms prior to absorption in the small intestine (see below). The monoglutamate forms of folate, including folic acid, are transported across the proximal small intestine. During the past decades, several attempts have been made to assess the bioavailability of folate polyglutamate compared to monoglutamate form, but the results were not consistent. It is suggested that bioavailability of ingested monoglutamates is significantly greater than that of folate polyglutamates apparently because of the requirement for hydrolysis of the latter (Gregory, 2001; Patanwala et al., 2014). It has been stated that the bioavailability of dietary folate is hampered by the polyglutamate chain to which most of the natural folate is attached. The polyglutamate chain must be removed by the enzyme conjugase present in the brush border of the small intestine prior to absorption and transport as a monoglutamate into the portal vein (Melse-Boonstra et al., 2002). Bioavailability of folate or folic acid is likely to be influenced by the amount ingested. Uncertainty exists as to the extent of folate bioavailability from natural food sources, as this is dependent on both host- and food-

related factors (McNulty & Pentieva, 2004). Limitations related to the host organism include folate status, health, age, gender, gastrointestinal function, and the use of medication and alcohol (Melse-Boonstra et al., 2002; Witthöft, 2011).

Food processing can also influence bioavailability by affecting the food matrix and folate stability, resulting in variable folate losses prior ingestion. Bioavailability of folates in food stuff has been difficult to assess quantitatively, but in general folates from animal products for human appear to be more bioavailable compared to plant-derived food (McKillop et al., 2003). By comparing homocysteine and glycine, both sensitive measures of folate status in the folate depleted–repleted rodent model, it was shown that the folate present in chicken egg in the form of 5-MTHF monoglutamate had the same or slightly greater relative bioavailability as crystalline folic acid (House, O'Connor, & Guenter, 2003).

2.4 Stability of Folates

Folates are susceptible to oxidative degradation during food processing which is enhanced by oxygen, light, and heat. Oxidation results in a splitting of the molecule into biologically inactive forms, of which *p*-aminobenzoyl-glutamate is one major form (Strandler, Patring, Jägerstad, & Jastrebova, 2015).The susceptibility of folate is largely influenced by the pH of the medium, reducing agents in the buffer, type of buffer, folate derivatives, and the food system. In most cases, folic acid shows considerably better stability than the reduced folates. The use of antioxidant agents such as ascorbic acid can improve considerably the stability of 5-MTHF (House, Braun, Ballance, O'Connor, & Guenter, 2002). Folate compounds are susceptible to light which is reported to cause cleavage at C^9–N^{10} position. Thereby, folate analysis should be often carried out in low light and glassware that prevents light penetration (i.e., wrapped with aluminum foil) (Indrawati et al., 2004).

2.5 Absorption and Folate Metabolism

After consumption of polyglutamate folates and synthetic monoglutamate, they undergo absorption in the intestinal tract. While folic acid can be absorbed directly, the polyglutamated forms of folates must be converted to their monoglutamate form. Within the intestinal tract of humans, this is principally achieved through the action of glutamate carboxypeptidase II (folate conjugase) located on the brush surface of enterocytes

(Visentin, Diop-Bove, Zhao, & Goldman, 2014). The monoglutamate forms of folate are then transported across the enterocyte via the action of specific transporters: (1) the reduced folate carrier or (2) the proton-coupled folate transporter (PCFT) (Said, 2011). The latter PCFT carrier is postulated to be the primary mechanism of folate transport in the upper intestinal tract (Visentin et al., 2014).

Following transport, monoglutamates are reduced to di- and tetrahydrofolate (THF) by reductase enzymes. THF is the parent compound of all biologically active form of folate. In next step, the THF is converted to 5,10-methylenetetrahydrofolate and 5-MTHF. From there, they are transferred to the hepatic portal vein which leads to the liver and systemic blood circulation and body tissues (Czeizel, Dudás, Paput, & Bánhidy, 2011; Visentin et al., 2014; Witthöft & Jägerstad, 2002).

After reduction to tetrahydrofolate, synthetic folic acid gains metabolic activity similar to other folate species. The enzyme dihydrofolate reductase is responsible in reducing folic acid to dihydrofolate (H_2folate) and next to THF (Fig. 3). The main function of folate in the human body system is the transport of one-carbon moieties, such as methyl and formyl groups. The 5,10-methylenetetrahydrofolate reductase enzyme is responsible for converting the THFs to 5-MTHF via 5,10-methylenetetrahydrofolate. 5-MTHF is the methyl donor in the remethylation of homocysteine to methionine through the action of methionine synthase (MS). Methionine (essential amino acid) will be converted to *S*-adenosylmethionine which is an important intracellular methyl group donor (Fox & Stover, 2008).

Folates act as a cofactor to transport one-carbon units at different oxidation levels (methyl-, formyl-, methylene-) for the biosynthesis of DNA and for hundreds of methylation reactions. Therefore, folates are important for all cell replication activities and restoring DNA and RNA. Folates play a role in regeneration of methionine from homocysteine. This reaction is catalyzed by MS and requires vitamin B12 as coenzyme. During this reaction, the methyl group in folate is transferred first from 5-MTHF to the cofactor to form methylcobalamin, and then the methyl group is ultimately transferred to homocysteine. Deficiency of vitamin B12 in cells results in the accumulation of 5-methylated folate, thus forming a block in folate metabolism, the "methyl trap" (Scott & Weir, 1993). Thus, vitamin B12 deficiency can create a conditional folate deficiency. Folate deficiency causes abnormal nuclear maturation in the blood cells which in turn leads to megaloblastic anemia (Aslinia, Mazza, & Yale, 2006).

2.6 Folate and Health

Beyond its role in normal metabolism, folate status has been linked to the development of chronic diseases, including cardiovascular disease, and also in reproductive health. Given the involvement of folate with other water-soluble vitamins in the methionine cycle, principally vitamin B6 and B12, specific roles for folate are often difficult to delineate. Folate deficiency has traditionally been recognized as a problem in countries struggling with poverty and malnutrition, with the main risk being macrocytic megaloblastic anemia (Gough, Read, McCarthy, & Waters, 1963). Low intake of folate associated with megaloblastic anemia led to a higher risk of giving birth to infants with NTDs and possibly other birth defects (Iyer & Tomar, 2012). With respect to chronic diseases, folate inadequacy has been linked to cardiovascular diseases (Graham et al., 1997), depression, Alzheimer's disease (Kruman et al., 2002), and cancer (Duthie, 1999). Folate plays a part in many metabolic pathways, including DNA replication, repair, and methylation, and in the synthesis of nucleotides, amino acids, and some vitamins. A dietary supply of this vitamin is therefore required to prevent nutritional deficiency in humans (LeBlanc, de Giori, Smid, Hugenholtz, & Sesma, 2007). Traditionally, folate deficiency was more common in populations that had a high intake of refined cereals and a low intake of green leafy vegetables and fruit. Other risk factors for folate deficiency include the malabsorption of folic acid, which can be caused by diseases affecting either intestinal pH or the jejunal mucosa, or due to genetic defects. Prior to the introduction of mandatory wheat flour fortification with folic acid in 1998, about 15% of adult women in the United States had low serum folate levels. Serum folate level is an indicator of recent dietary folate intake, and the most widely used method of assessing folate status (Pfeiffer, Caudill, Gunter, Osterloh, & Sampson, 2005).

The main dietary sources of folate are green leafy vegetables, pulses, liver, egg, and fortified grain products. As such, the requirement for this vitamin is supplied mainly via the dietary intake of folates and the use of synthetic folic acid. Vegetables and pulses are considered as rich sources of folate having 200–600 $\mu g\ 100\,g^{-1}$. Citrus fruits and cereals are good sources of folate with concentration of 50–100 $\mu g\ 100\,g^{-1}$. Egg yolk has high content of folate for about 90 $\mu g\ 100\,g^{-1}$. In addition to dietary sources of folate, either from natural or supplemental/synthetic forms, the human digestive tract harbors microorganisms capable of synthesizing folate. Evidence is accumulating

that this source of folate is metabolically available to the host (Aufreiter et al., 2009). However, the extent of this contribution has not been sufficiently quantified to date.

2.6.1 Neural Tube Defects

NTDs (spina bifida, encephalocele, and anencephaly) represent a major group of birth defects. During fetal development, the neural tube closes by the third week of postconception in humans. Failure of the neural tube to close completely results in a neural tube defect or NTD (Czeizel & Dudas, 1992). Maternal nutrition factors contribute significantly to the various etiologies of NTDs. Previous investigation (Smithells, Sheppard, & Schorah, 1976) reported that the diets and postnatal blood of women who had given birth to babies with an NTD were lacking in several micronutrients, principally folic acid. Following two decades of research, it was established that folic acid supplementation reduces 50%–75% of NTD-affected pregnancies (Laurence, James, Miller, Tennant, & Campbell, 1981; Scott & Weir, 1993; Seller & Nevin, 1984; Vergel, Sanchez, Heredero, Rodriguez, & Martinez, 1990). Dietary supplementation with 400 mg of folic acid per day showed 70% prevention of NTD in women with a past history of carrying a fetus with NTDs and 50% of NTDs in the general population. It was found that 30%–50% of the population is nonresponsive to maternal folate level, demonstrating folate-responsive and nonresponsive individuals in the human population (Berry & Li, 2002; Czeizel et al., 2011). The latter observations provided strong evidence that additional folate might provide a public health benefit, particularly with respect to healthy birth outcomes.

2.7 Folate Fortification

Food fortification refers to addition of micronutrients into processed foods. It is a valid technology to improve micronutrient levels, as part of food-based approaches, if access to adequate levels of specific nutrients is limited. Supplementation, on the other hand, refers to the provision of micronutrients in the form of pills, capsules, or syrups. Typically, supplementation is considered to be a rapid approach to achieve enhanced intakes of nutrients of concern, in stable and bioavailable forms, in select population groups. However, with respect to NTDs, given the fact that 3 weeks postconception reflects the maximum time for the protective effect of folate, challenges to the efficacy of folic acid supplementation for NTD risk mitigation exist. These include the fact that many pregnancies are unplanned and the usage of supplements by women of child-bearing age is typically below 40%. In a cohort

of young, college-aged women, only 26% of the participants reported taking a supplement containing folic acid (Shuaibi, House, & Sevenhuysen, 2008). Supplement usage by women during pregnancy is, however, considerably higher, with greater than 90% of a cohort of pregnant women in Ontario, Canada, taking supplements with folic acid (Plumptre et al., 2015). However, this level of supplement usage may not be NTD-protective given the need for adequate periconceptional folate status. Fortification of the food supply with folic acid presented an alternative approach to ensuring adequate intakes.

In 1992, to reduce the number of cases of NTDs, the US Public Health Service recommended that all women capable of becoming pregnant consume 400 μg of folic acid daily. Three approaches were advocated to increase folate consumption, including the improvement of dietary habits to ensure the consumption of foods naturally rich in folate, fortify foods with folic acid, and promote the use of dietary supplements containing folic acid (CDC, 1991). However, for reasons discussed above, the governments of the United States and Canada, along with several other countries, introduced the mandatory fortification of grain products with folic acid (FDA, 1996). Crystalline folic acid (monoglutamate form) was chosen as the form of folate to use in fortifying foods owing to its availability, cost, and stability.

Mandatory fortification of cereal grain products started in 1998 from United States and followed by Canada and some Latin American countries but not in Norway or in the United Kingdom (Wien et al., 2012). Thereafter, the results of several studies and surveillance show the number of NTD-affected pregnancies declined by as much as half. Given the significant decline in NTD rates, mandatory folic acid fortification became recognized as a major public health triumph (De Wals et al., 2007). However, in recent years, concern has been raised that populations within folic acid-fortified countries might be exposed to excessive levels of this form of folate.

2.8 Adverse Effects of Folic Acid Food Fortification and Supplementation

Despite documented beneficial effects of folic acid fortification on preventing NTDs, concern has been raised about the amount of population exposure to crystalline folic acid. The potential for folic acid, enhanced via food fortification, in masking vitamin B12 deficiency has been raised, as the latter leads to the progression of irreversible neurological symptoms (Molloy, Brody, Mills, Scott, & Kirke, 2009). The latter findings were of particular importance to those individuals who received folic acid from both supplements and a range of fortified foods because in this situation people

may exceed the upper limit for folic acid intake (1 mg from folic acid). Furthermore, there are some reports on the relation between folic acid supplementation during pregnancy and risk for miscarriage or an increase in the occurrence of multiple births (Czeizel & Dudas, 1992).

Another concern that has been raised in relation to increased exposure to dietary folic acid relates to the potential for increased risk of gastrointestinal cancer, including colorectal cancer (Kim, 2004). A systematic review found only prostate cancer to be linked to high folic acid intakes (Wien et al., 2012). While the latter finding may not translate to specific concerns related to maternal folate exposure, recent preclinical studies in animals have shown that high folic acid (and other methyl donor) intakes may lead to epigenetic changes in offspring, increasing the risk of development of diabetes and alterations in food intake behavior (Huot et al., 2016). Perhaps it is time to reevaluate the strategy of fortifying foods with folic acid, taking into account the impacts to the entire population and not just a subsection at risk for NTD-affected pregnancies.

2.9 Replacing Synthetic Folic Acid With Natural Folate

The introduction of mandatory folic acid fortification of grain products in certain jurisdictions, including Canada and the United States, has succeeded in decreasing the rates of NTD by 20%–50% (Persad, Van den Hof, Dubé, & Zimmer, 2002; Ray, 2004). However, in some countries like the Netherlands, folic acid fortification is prohibited due to growing debate about the long-term safety of folic acid consumption by general population (Varela-Moreiras, Murphy, & Scott, 2009). The debate addresses two main risks of long-term consumption of high folic acid intake which could promote the formation of colorectal tumors (Kim, 2004; Mason et al., 2007) and masking the appearance of vitamin B12 deficiency anemia (Morris, Jacques, Rosenberg, & Selhub, 2007, 2010). Recent studies regarding folic acid fortification are rediscovering the harms to the nervous system from long-term exposure to doses of folic acid between 0.5 and 1 mg in the presence of vitamin B12 deficiency (Reynolds, 2016). Thereby, it is highly recommended to review the safe upper intake level of folic acid to prevent the health risk factors.

As discussed above, synthetic folic acid is absorbed by carrier-mediated mechanism, and it must be reduced and then metabolized to 5-MTHF in the human mucosal cell and/or liver in order to be metabolically active. Because

the ability of conversion is limited, unmetabolized folic acid can appear in the systemic circulation, even after low-dose application (Pietrzik et al., 2010). Due to the suspected adverse effects of folic acid fortification, attention has been directed toward other solutions that might help increase population intake of natural folates, which do not carry with them the same safety concerns as synthetic folic acid, particularly with respect to the masking of vitamin B12 deficiency. As such, other strategies have been investigated in order to increase population intake of natural folates without adverse effect of synthetic folic acid. The development of novel foods enriched with natural folates has been considered as an innovative solution in nutritional strategies.

One strategy for increasing natural folates in the food supply includes the development of novel functional foods based on animal products enriched with natural folate through the addition of high dose of synthetic folic acid to animal feed (Altic, McNulty, Hoey, McAnena, & Pentieva, 2016; House et al., 2002). Research has shown that hen eggs can be enriched with folate by supplementing hen's diet with folic acid and reaching maximum folate content of about 2.5 times that of a normal egg (Altic et al., 2016; House et al., 2002; Sahlin & House, 2006). Natural folates in novel folate-enriched eggs are highly stable with little or no losses during storage at refrigerator or room temperature for 27 days (Altic et al., 2016). Studies revealed that the folate which appears in enriched eggs is in the natural form, mainly as 5-MTHF, and this strategy could offer an efficient solution to increase folate intake of population, especially where long-term consumption of folic acid-fortified foods has been criticized (Hoey et al., 2009). This researcher stated that no polyglutamate forms were present in egg yolk. House et al. (2003) also reported the relative bioavailability of folate in eggs is higher or equal to 100% compared to folic acid. The 5-MTHF in egg yolk does not oxidize during cold (4°C) storage for 4 weeks and folate levels remained constant relative to freshly collected eggs (House et al., 2002).

Compared to green leafy vegetables, folates in animal products including eggs are also usually more stable during thermal treatment (McKillop et al., 2003). However, a significant obstacle in egg consuming is the perception that egg consumption is associated with a rise in blood cholesterol levels. Food processing techniques such as fractionation methods enable scientists to fractionate egg yolk in different components with different compositions. Fractionation of egg yolk by centrifugation techniques can enable us to separate a low-cholesterol content fraction of yolk which is concentrated with

5-MTHF (Naderi, House, & Pouliot, 2014). The consumption of these novel folate-enriched eggs or egg-derived, folate-enriched fractions could represent alternative and cost-efficient ways of increasing folate intake and potentially folate status of population in countries that mandatory folic acid fortification remains nonexistent (Altic et al., 2016). Another way to introduce a stabilized form of 5-MTHF into the food supply is via direct encapsulation (Liu et al., 2012), a strategy shown to lead to effective fortification of cereal grain products, including noodles and bakery products (Liu, Green, & Kitts, 2015; Liu, Green, Wong, & Kitts, 2013).

Another strategy to increasing the supply of natural folates is via encapsulated probiotics (Divya & Nampoothiri, 2015). The use of folate-producing food-grade microorganisms has been positioned as a natural alternative to fortification with the synthetic folic acid (Laiño, Zelaya, del Valle, de Giori, & LeBlanc, 2015). Fermented milk bioenriched with folate was produced from folate-producing lactic acid bacteria, and this product effectively increased plasma folate concentrations (Laiño et al., 2015). The latter method represents an approach toward biofortification of foods with folate. The use of genetically modified biofortified crops offers additional opportunities. Researchers applied transgenic breeding techniques in order to enhance vitamin or mineral concentrations in some crops such as Golden Rice (De Steur, Feng, Xiaoping, & Gellynck, 2013). However, the genetically modified biofortification faces challenges for commercialization, due to regulatory hurdles and consumer acceptance of genetically modified foods. Folate biofortification through breeding or metabolic engineering in rice, tomato, potato, wheat, and cassava was assessed; however, this strategy needs to be developed to enable adequate biofortification of other staples (Strobbe & Van Der Straeten, 2017).

3. SUMMARY

Folate is a water-soluble vitamin critical for optimal health in humans. Recent attention has focused on the supply of folic acid to the population, with particular emphasis on the reduction of the incidence of NTD rates in newborn infants. Recent evidence is challenging current public health strategies with respect to food fortification, necessitating a review of the current approaches. Innovative alternative approaches to supplying folate to the human population include the use of biofortified foods and novel, stabilized forms of folate.

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