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RESEARCH ARTICLE

Folate and Folic Acid Metabolism: A Significant Nutrient-Gene-Environment Interaction

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ABSTRACT

Folate has an important metabolic role by providing one carbon units that are used for nucleotide biosynthesis and methylation reactions that are both vital for epigenetic control, genomic stability and the maintenance of health.

Important not only for its availability as an essential nutrient but folate also intertwines us firmly within the environment as part of an unexpected link between light and the genome.

Folate deficiency has been overcome by replacement with folic acid, taken as a supplement and/or through food fortification but is this solving one problem but risking others with the wider long-term implication of this manipulation in its potential to alter the human genome.

The mandatory folic acid food fortification public health policy and implementation needs to be re-examined and possibly debated in the public arena, particularly in view of the risk of altering the human genome. Have the public been adequately informed and are there implications for the whole vitamin and mineral supplement industry?

Introduction

Folate, from the Latin Folium (leaf), is a generic term for the related family of water-soluble B-group vitamins found widely in food-stuffs. The structure consists of a pteridine ring attached through a methylene group to a p-aminobenzoic acid and glutamate residue of variable length¹(Figure 1). Folate metabolism mediates the transfer of one carbon units for use in biochemical reactions vital to human physiology and health². The diversity of reactions that involve the one carbon units results from the ability of the coenzyme tetrahydrofolate to carry activated one carbon units at several different oxidation states and from the ability of the cell to readily interconvert these forms. This includes nucleotide synthesis and involvement in the synthesis of S-adenosylmethionine which acts as a methyl group donor for methylation reactions of critical importance in epigenetic gene expression and DNA stability. It is also involved in de novo synthesis of purine and thymidylate for use in DNA replication and repair³. Abnormalities in folate metabolism can lead to the development of carcinogenesis^{4,5,6}. Abnormal folate stasis is implicated in a number of other diseases. Congenital abnormalities such as neural tube defects and pregnancy complications, vascular disease, neurodegenerative and psychiatric disorders. Interaction with other B group vitamins can also have an influence on cognition in the elderly.

Native folate is available through a wide range of food stuffs and with a well-balanced diet of fresh foods adequate folate should be obtainable to populations within the Western world, at least. Unfortunately, even with modern, rapid international trade and movements of fresh foods within individual countries, widely available at a reasonable price, there remains small groups within the community that may receive inadequate nutrition. Is mandatory food fortification the best way to deal with this situation? Folate deficiency is particularly crucial around conception and early

pregnancy and this warrants particular consideration.

Native folate and folic acid are very similar as will be seen with their absorption and transport but there is a difference. Unique to humans within other mammalian species, the human liver enzyme, dihydrofolate reductase has a reduced activity and poor ability to reduce folic acid. This has resulted in unmetabolized folic acid appearing in the plasma in populations where mandatory fortification has been introduced. This is particularly prominent in the United States where the situation is exacerbated by widespread use of vitamin supplements. This may have long term health implications.

Absorption and transport

The human source of folate is as dietary reduced methyl and formyl polyglutamates available through a wide range of food stuffs. They are absorbed at the mucosal epithelial cell brush border in the proximal small intestine (jejunum) by a saturable, pH and energy dependent transport mechanism after first being hydrolysed to a monoglutamate, the form that circulates in the blood. Once absorbed into tissues they are converted back into polyglutamates which helps retain them within the cell and also act as better substrates for subsequent biochemical reactions.

Folic acid (pteroylmonoglutamic acid), though rare in nature, is the most oxidised and stable form of the vitamin and is used extensively for supplementation and food fortification, having similar nutritional properties and chemical structure to natural folates. The intestinal transport mechanism has similar affinity for both oxidised and reduced folate forms. However, oral doses of folic acid in excess of 260-280µg have been reported to lead to the appearance of untransformed folic acid in the systemic circulation⁷. This may have implications for the introduction of mandatory fortification of food stuffs. It indicates a saturation point and evidence that intestinal conversion is not a prerequisite for transport.

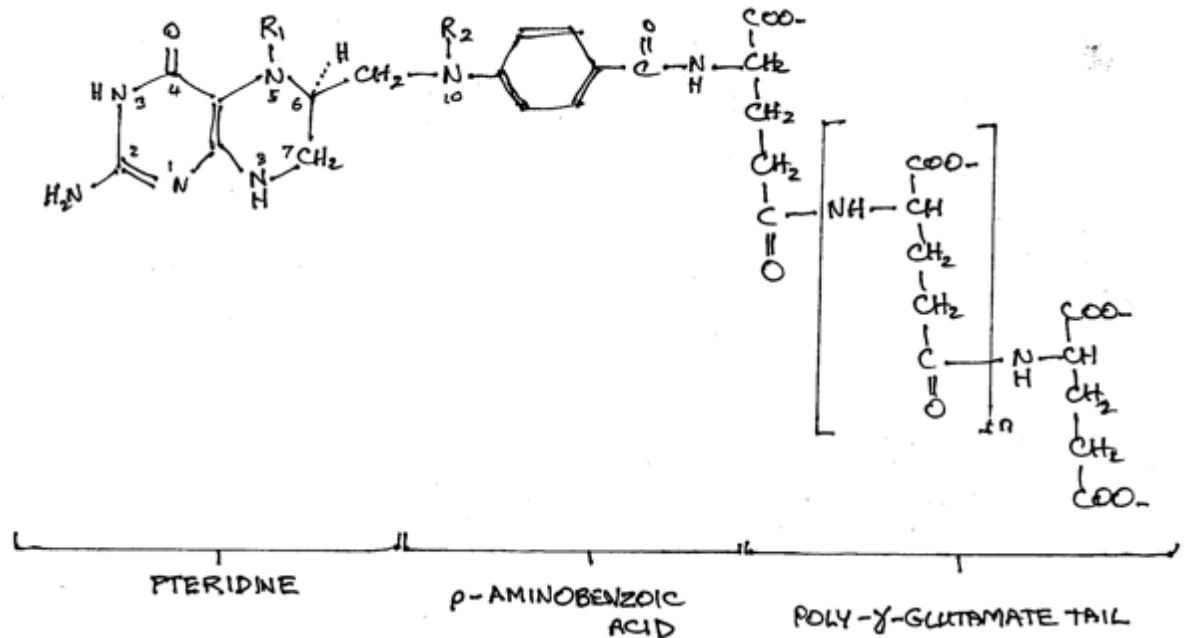


Figure 1. The structure of tetrahydrofolate.

Activated one carbon units are carried at N5 and N10 (R_1 and R_2) and can be taken as the division between physiological and non-physiological oral doses of folic acid. The absorbed folate may undergo biotransformation in the mucosa and then is transported via the hepatic portal vein to the liver where an extensive amount is removed (liver first-pass)^{8,9}.

The liver has a higher affinity for removal of folic acid but lower for 5-methyltetrahydrofolate (CH_3 -THF), allowing a fraction to proceed to the systemic circulation. CH_3 -THF being the prominent plasma form, under fasting conditions, for passage to the tissues. Folates removed by the liver, some of which may have undergone further biotransformation, are partially released into the bile allowing significant reabsorption from the small intestine and subsequent delivery of CH_3 -THF to the tissues (enterohepatic recirculation), for maintenance of baseline folate levels¹⁰. It has been suggested that the initial site of folic acid biotransformation in humans is the liver rather than the mucosa, due to the almost complete absence of CH_3 -THF response in the portal vein to an oral dose of folic acid¹¹. Humans are unique amongst mammals and birds in having a reduced dihydrofolate reductase activity and poor ability to reduce folic acid, so even a moderate 'physiological dose' could eventually saturate the preliminary liver folate-monoglutamate pool with the appearance of unmetabolized folic acid in the systemic circulation.

Folate metabolism

The metabolic role of folate is to carry one-carbon units at various levels of oxidation. There are a number of one-carbon transfer reactions that occur within the cell. Of significance are the conversion of serine to glycine, catabolism of histidine and the synthesis of thymidylate, methionine and purine. These reactions occur through electron transfer facilitated by specific enzyme and coenzyme systems.

The liver is the body's main store of folate. The folate circulates in the plasma as 5-methyltetrahydrofolate (CH_3 -THF) and largely enters the cell in this form. Methionine synthetase catalyses the demethylation of CH_3 -THF to tetrahydrofolate (THF), the rate limiting step in cellular accumulation of folates since it is the only enzyme capable of this demethylation. THF is the optimum substrate for polyglutamylation. Polyglutamates provide better cellular retention but also K_m values decrease with increasing oligo- γ -glutamyl chain length. Many folate enzymes are multifunctional and channel carbon one units without reaching equilibrium, allowing channelling of the substrates between enzymes in a way which controls biosynthetic pathways¹².

The principal origin of one-carbon units in folate-dependent one-carbon metabolism is the β -carbon of serine in a reaction catalysed by serine hydroxymethyl transferase. Glycine is generated and THF is converted to 5,10-methylenetetrahydrofolate (CH_2 -THF).

Possibly the most important role of folate is purine and pyrimidine nucleotide biosynthesis. CH₂-THF methylates deoxyuridylate monophosphate (dUMP) to form thymidylate monophosphate (TMP), catalysed by thymidylate synthetase in the synthesis of pyrimidine nucleotides and is the rate limiting step. Expression of thymidylate synthetase peaks

during S phase of the cell cycle and is related to replication rate. Increased thymidylate synthetase actively in proliferating cells elevates DHF levels. DHF inhibits MTHFR providing a regulatory mechanism for ensuring priority is given to nucleic acid synthesis over methionine formation. Figure 2.

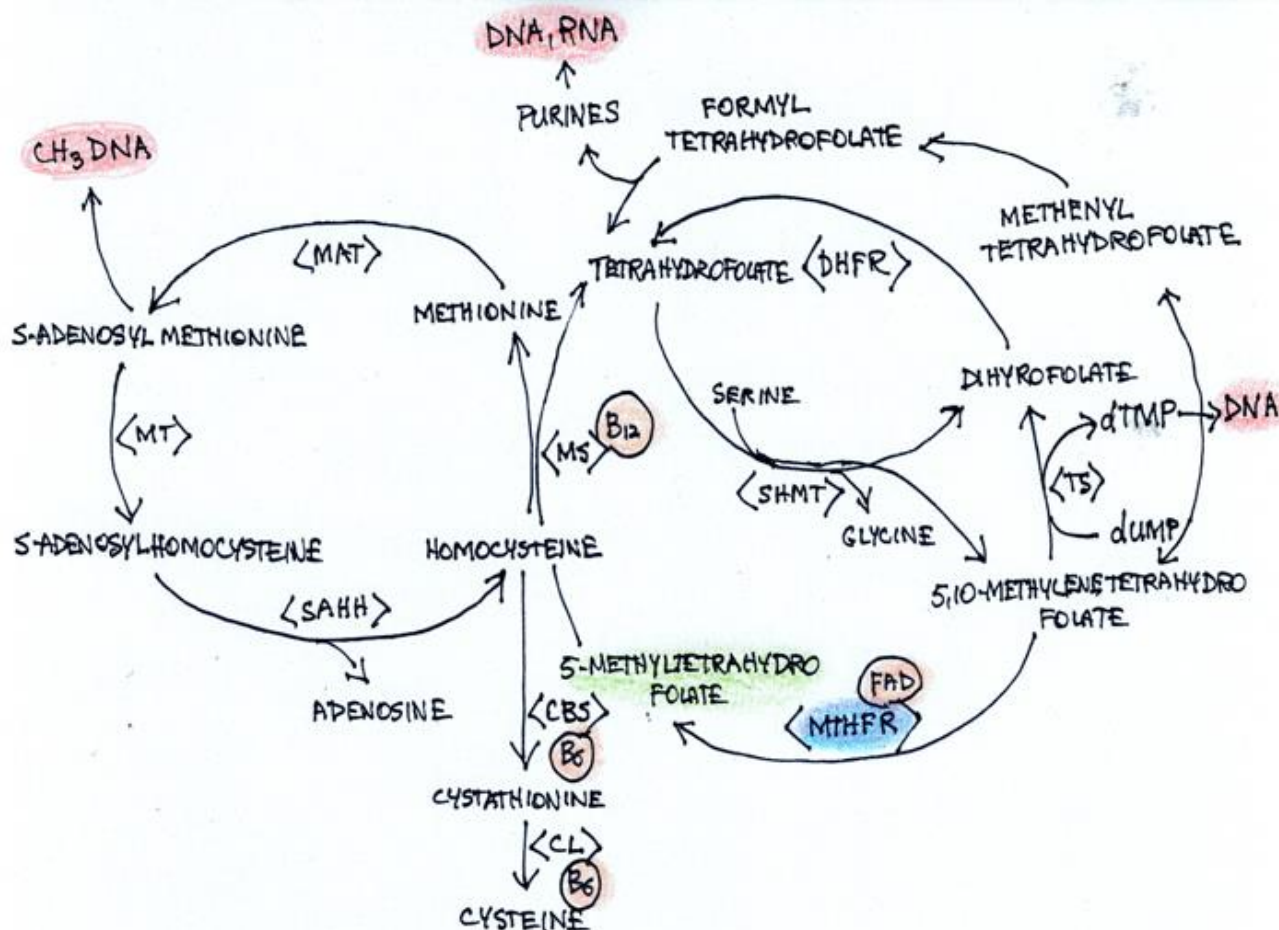
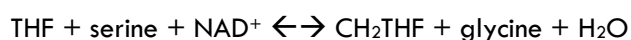


Figure 2. Folate and homocysteine cycles.

Homocysteine (Hcy) is formed from S-adenosylhomocysteine. Re-methylation to methionine is catalysed by methionine synthetase which requires Vitamin B₁₂ as a cofactor and 5-methyltetrahydrofolate (CH₃-THF) as a substrate. CH₃-THF is formed by the action of the flavin adenine dinucleotide (FAD)-dependent enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) which sits at a crucial metabolic nexus whereby the folate pool can be directed to homocysteine re-methylation, S-adenosylmethionine and on to DNA methylation, an important factor in carcinogenesis. This is at the expense of an alternative pathway directing the folate pool to

DNA and RNA biosynthesis. CBS, cystathionine β synthetase; CL, cystathionine lyase; DHFR, dihydrofolate reductase; MAT, methionine adenosyl transferase; MS, methionine synthetase; MT, methyltransferase; SAHH, S-adenosylhomocysteine hydrolase; SHMT, serine hydroxyl methyl transferase; TS, thymidylate synthetase. Serine hydroxymethyl transferase (SHMT) catalyses the reversible interconversion of serine and glycine. The reaction requires Vitamin B₁₂ as a coenzyme and introduces the β carbon of serine into the one-carbon pool at the CH₂O level of oxidation:



CH₂THF is a crucial intermediate coenzyme state. Although CH₂THF is used by MTHFR to produce CH₃THF in an irreversible step which then requires B₁₂-dependent methionine synthetase to cycle Hcy into methionine, it is also required for both thymidine synthetase and methylenetetrahydrofolate dehydrogenase in the synthesis of DNA thymine and purine.

Thus, CH₂THF sits at the cross-roads where three important pathways intersect and its production by SHMT is an important step in human one-carbon metabolism. Figure 2.

Hcy sits on the intersection of two important pathways and its production by SHMT is regulated by several enzymes. Separation of Hcy between *de novo* methionine biosynthesis and transsulphuration to cystathionine is regulated by S-adenosylmethionine (SAM).

Compartmentalisation of folate-related one-carbon metabolism

It is now recognised that the mitochondria participate in folate-mediated one-carbon metabolism. Mitochondria are a site of oxidation of one-carbon donors, such as serine, glycine, sarcosine and dimethyl glycine, with participation by folate coenzymes and folate-dependent enzymes in these mitochondrial processes. A model has developed with interdependent but parallel cytological and mitochondrial pathways and connected by one-carbon donors.

Mitochondrial and cytological pools differ in distribution of specific forms of folate cofactors and the extent of their polyglutamylation. Serine derived one-carbon units flow, mostly unidirectionally, through the mitochondria in the direction of CH₂THF to 10 formyl-THF to formate and onto methionine. The cytological and mitochondrial redox states exert considerable control over metabolic processes¹³ but it is not yet understood how one-carbon fluxes respond to redox state.

In the nucleus thymidylate synthetase (TS) catalyses methylation of deoxyuridylate (dUMP) to thymidylate (dTMP) using CH₂-THF as a donor. This reaction is unique in folate one-carbon transfers in that the THF carrier is oxidised to DHF with electrons used to reduce the one-carbon unit to the methyl level. To re-enter the active pool DHF must be reduced back to THF catalysed by dihydrofolate reductase (DHFR). Serine hydroxymethyl transferase (SHMT), TS and DHFR are all translocated into the nucleus during S and G₂/M phases after modification by small ubiquitin-like modifier (SUMO). These three enzymes constitute a dTMP synthesis cycle in which serine serves as the one-carbon donor through the SHMT reaction.

Ultraviolet radiation has been shown to increase SHMT translation, accompanied by increased nuclear localisation of the *de novo* thymidylate biosynthetic pathway and a decrease in strand breaks, indicating a role for SHMT and nuclear folate metabolism in DNA repair¹⁴.

Methylenetetrahydrofolate reductase polymorphism

C677T polymorphism in the *MTHFR* gene results in a phenotype characterised by reduced catalytic activity of the enzyme, affecting folate distribution and elevated homocysteine (Hcy) under conditions of impaired folate status¹⁵. It has been consistently found that the T allele is associated with high concentrations of plasma Hcy. The effect is most pronounced in the homozygous TT genotype combined with low folate concentrations¹⁶. Prevalence is related to ethnicity. The homozygous TT genotype represented in 10% of Caucasians and 20% in some Italian populations¹⁷. High concentrations of Hcy are associated with neural tube defects and colorectal neoplasia. Folic acid supplementation 0.5-2.0mg daily decreased Hcy in TT subjects who obtained the same Hcy concentration as CC genotypes¹⁸. It was reported that in TT subjects formylated THF polyglutamates accumulated at the expense of CH₃THF, the prominent species in the CC genotype, causing retention of folate species committed to purine/pyrimidine synthesis.

It has been shown that the concentration of Vitamin B₂ (riboflavin) is inversely proportional to the plasma concentration of Hcy with either high or low concentrations of serum folate but confined to the C677T phenotype²⁰. A possible explanation of riboflavin regulation here could relate to FAD as a dependent cofactor in MTHFR function, the transition affecting FAD dissociation kinetics²¹.

Serum Vitamin B₁₂ is an established determinant of, and also inversely related to plasma Hcy, most pronounced in the TT genotype, but the relationship remains even after folate supplementation²².

An investigation of subjects with high Hcy (>40 µmol/L), these individuals representing the upper 0.4% of the Hcy distribution from a general population sample of 18,000 men and women. It found 70% were TT genotype with folate deficiency. Additionally, they were more frequently heavy smokers, drank more coffee and had a sedentary life-style compared to CC and CT genotypes^{23,24}. This study suggests that an unhealthy life-style can contribute to markedly elevated Hcy in combination with the TT genotype.

It has also been found that the TT genotype is associated with lower DNA methylation in peripheral leukocytes as compared with the CC

genotype, thought to be due to reduced availability of CH₃-THF for S-adenosyl methionine biosynthesis²⁵. This study demonstrated that altered folate distribution has secondary metabolic effects beyond hyper-homocysteinemia, DNA methylation being a significant factor in carcinogenesis²⁶.

Low folate is a risk factor for colorectal cancer, as well as some other forms of cancer²⁶.

Adequate dietary folate in the TT genotype has demonstrated a 50% risk reduction. Whereas, low folate offers no protection and probable increased risk^{27,28,29}. Protection may relate to availability of DNA precursors (purine/pyrimidine) for DNA synthesis, with efficient repair and avoiding uracil incorporation. TT genotype and low folate impairs Hcy re-methylation leading to DNA hypomethylation, a known carcinoma risk³⁰.

Studies identified two high risk categories for premalignant colorectal adenomas-smokers with a combination of low folate and TT genotype and high folate combined with the CC genotype³¹. This has significance in the debate over mandatory folic acid fortification of food-stuffs with the Aspirin-Folate Prevention Trial suggesting that folic acid supplementation may increase the risk of multiple colorectal adenomas³².

The prevalence of this polymorphism and the TT variant in most populations may represent an ancestral genetic adaptation to living constraints, such as injury or vitamin-poor diet more prevalent in early hunter-gatherer life-styles but now having become a determinant of modern disease profiles³³.

Folate chemistry and the environment

Folate chemistry is attuned to the environment. It is deeply embedded in several evolutionary and developmental processes that have light as an environmental effector, making it more than a simple carrier of one-carbon units for biosynthetic reactions. UVR labile folate and light-dependent hormone vitamin D can both be implicated in the evolution of human skin pigmentation and depigmentation, respectively³⁴⁻³⁶. Although there is also multifactorial involvement of genes in the process (MC1R, MATP(SLC45A2), OCA2, TYRP, KIT, PPAR, DRD, EGFR)³⁷⁻³⁹. The deleterious effects of UV radiation on DNA integrity and function are well recognised. Thymine bases are converted to thymidine dimers and photoproducts by UV light exposure, inhibiting transcription⁴⁰. Excision repair processes can correct the damage but require folate to ensure fidelity of DNA-dTMP biosynthesis, used in the repair process⁴¹. It has also been demonstrated that there is an increase in translation of the enzyme SHMT and translocation to the nucleus, in response to UV radiation, to be

incorporated into the *de novo* thymidylate biosynthetic pathway, decreasing strand breaks and indicating a role for SHMT and nuclear folate metabolism in DNA repair. An evolutionary adaptive response to UV radiation exposure⁴². UVB/near UVA at 312nm augments oxidation of CH₃THF the plasma and cellular form of folate, to render oxidised CH₃DHF, with irreversible loss of vitamin activity (C⁹-N¹⁰ bond scission)⁴³. This effect is reduced in the presence of the antioxidant, Ascorbic acid. CH₃.THF photodegradation is enhanced by endogenous photosensitisers found alongside it in the blood. Both UVA and visible light can cause oxidation of CH₃-THF in the presence of Vitamin B₂ (riboflavin) and uroporphyrin, whereas bilirubin can be photoprotective⁴⁴.

Light and the human life cycle

Contemporary society has stepped away from our ancestral genes in terms of circadian disharmony and obesogenic life-styles inducing earlier onset of chronic degenerative diseases. Despite these changes the genome and environment, however, are still tightly interactive around conception. For example, ultraviolet light attenuating the maternal immune system leading to cytokine production that influences the foetal genome⁴⁵. In 1991 a high initial load hypothesis was presented. It proposed that early development of biological systems, and more specifically the human foetus, produces an exceptionally high load of initial damage, which is comparable with the amount of subsequent aging-related deterioration accumulating during the rest of the entire adult life. This suggests that early-life events may affect survival in later adult life through the level of initial damage⁴¹. A study by Gavrilov and Gavrilova tested predictions from this hypothesis, and confirmed that early-life indicators such as maternal age at conception and month of birth affected survival in later adult life, providing support for the idea of foetal origins of adult degenerative diseases and early-life programming of aging and longevity⁴⁶. A study by Lowell and Davis explored the relationship between season of birth and human life-span. They found that there was a relationship to cycles of solar radiation. These cycles give rise to periodic rise and fall in intensity of solar radiation with an approximate period of 11 years. Sunspot activity and solar magnetic storms are considered a proxy for intensity of solar radiation. They found that those born in peaks of solar cycles lived an average of 1.5 years less than those born in non-peak years. A similar analysis for month of birth and the pattern of peak and non-peak life-span differences was nearly identical to the pattern of season variation in light-intensity⁴⁵. In other words, early human embryological

development is responsive to photoperiod and oxidative stress with an influence on long-term disease incidence⁴⁷. This relationship persists despite the apparent isolation of modern life-style from seasonal influences, such as temperature, photoperiod and nutrient availability⁴⁸.

The influence of the C677T-MTHF genotype on human health has already been demonstrated but the day length experienced during the peri-conceptual period predicts the C677T-MTHF genotype⁴⁹. A clear example of foetal origins of adult disease^{50,51}. Hypotheses in relation to this include; dermal destruction of CH₃THF leading to lower CH₂THF status^{43,34} which, in the embryo, might increase TT genomic viability and hence the mutant T frequency, or altered seasonal availability of food folates genetically buffering negatives influences of the mutant T allele, or both. Also, seasonal oxidative stress with negative effects on the neuroendocrine axis⁴⁹.

Food folates Vs folic acid

Peri-conceptual folic acid supplementation has markedly reduced the occurrence of neural tube defects which have been related to maternal low blood folate and elevated Hcy concentrations⁴³. However, there are a number of concerns that have been raised in relation to the use of folic acid for food fortification, particularly bioaccumulation due to the limited ability of human liver dihydrofolate reductase to convert the folic acid to CH₃THF⁵². Fortification will raise the concentration of total folates, not just unmetabolised folic acid, above that occurring with normal diets. This is exacerbated by the increasing use of multivitamin supplements. Serum folate >45mmol/L are considered supraphysiologic. These levels were found in 23% of the US population after fortification, including 43% of children

< 5yrs and 38% of the elderly⁵³. Potential adverse effects include:

Masking of B₁₂ deficiency anaemia with negative effects on cognition in the elderly. Theoretically, excess folate with low B₁₂ could bypass the metabolic block in nucleic acid synthesis allowing cell division in the marrow to continue, creating an increased demand for methyl groups by growing cells with further depletion of methylation potential, particularly in non-dividing cells in the nervous system⁵⁴. After fortification in the US, a study found a cohort of elderly with high folate and low B₁₂ to be approximately 4% of the study group⁵⁵. This is not an insignificant number at risk of anaemia and cognitive impairment.

Dual role in cancer.

Animal studies on colorectal cancer have shown timing and dose of folate interventions are critical.

If folate supplementation is stated before the establishment of neoplastic foci, there is suppression of the tumour, but if stated after establishment, it enhances tumour progression. Thus, there may be protection against initiation but facilitation in growth of pre-neoplastic cells. In humans, any effect of folate on carcinogenesis will interact with a large number of risk factors and the pattern of these risk factors will differ between individuals.

Adverse effect on anti-folate therapy

Drugs designed to interfere with folate metabolic pathways are widely used in medicine, treating cancer, rheumatoid arthritis, malaria, psoriasis and ectopic pregnancy. Antifolate drugs like methotrexate resemble DHF and inhibit the enzyme dihydrofolate reductase (DHFR) which converts DHF to THF. TMP formation is sensitive to depressed levels of THF, leading to inhibition of DNA synthesis in rapidly dividing cells.

Reduced formation of natural killer cells

A study of natural killer cell (NK) cytotoxicity in post-menopausal women in the US after fortification found that although there was no relation between total plasma folates and NK cytotoxicity, there was a highly significant inverse linear association between the amount of unmetabolised folic acid in the plasma and NK cytotoxicity⁵⁶. These findings raise the hypothesis that excess folic acid from supplements or fortification could impair normal immune function.

Increase in multiple births

A study of women undergoing in vitro fertilisation has found a positive relation between blood folate concentration and the incidence of twin births⁵⁷. Twin births have a higher risk of complications for both mother and child. Is fortification reducing one pregnancy risk but replacing it with another?

Can maternal folate status influence foetal genotype?

In Spain, the prevalence of the TT genotype in the population has approximately doubled since the introduction of peri-conceptual folic acid supplementation in the 80's⁵⁸. The authors speculate that the infants with the T allele normally have a higher rate of spontaneous abortion due to higher maternal homocysteine levels. Supplementation stabilises the MTHFR enzyme lowering homocysteine levels, reducing risk of abortion and increasing the proportion born with the T allele.

Conclusion

We, as humans, tend to see ourselves as the superior beings, having risen above the struggle for survival that all other living organisms are captive to within a capricious environment. The story of folate demonstrates how tightly bound to our

genetics, the environment, and particularly, the influence of the sun in our survival as a species.

We have arisen from the same building blocks as are all other living organisms on the planet and are still under the influence of its developmental and evolutionary forces.

We isolate ourselves from the harsh realities of the environment through urbanisation and technological advances but our future is still tightly interwoven into

the fabric of life in ways that are not always immediately obvious.

Government intervention through food fortification attempts to overcome deficiencies in nutrition that maybe a public health problem in small sub-groups within the community, and this is obviously done with good intentions, but is this in the best interests of the community as a whole and have the consequences been fully examined and understood?

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