

SYSTEMS BIOLOGY, FUNCTIONAL MEDICINE, AND FOLATES

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Human biology is often not as simple as it seems on the surface. This is certainly the case with folates and their relationship to disease prevention. During the decades from the 1960s through the 1980s, Smithells made the discovery that folic acid was important in the prevention of neural tube defects in babies,¹ Butterworth made the observation that folic acid was important in the prevention of cervical dysplasia,² and McCully reported that folic acid was an important nutrient for the prevention of atherosclerosis associated with elevated homocysteine.³ These 3 observations redefined the clinical importance of folate nutrition from that of a vitamin that was important in hematology for the prevention of anemia to a state of importance in many subspecialties of medicine from obstetrics and gynecology to neurology and cardiology. More recently, epidemiological evidence has associated low-folate diets with an increased risk of colorectal neoplasia and colorectal cancer.⁴

STUDIES HAVE UNEXPECTED OUTCOMES

The interest in folic acid as a potential chemopreventive agent for colon cancer resulted in a recent multi-centered clinical intervention trial.⁵ The double-blind, placebo-controlled, randomized trial was conducted at 9 clinical centers between 1994 and 2004. The study population was composed of 1021 men and women with a history of colorectal adenomas with no previous history of large intestine carcinoma. The 516 participants randomized to the treatment group received 1000 µg of folic acid daily as a food supplement. The primary outcome measure of the study was occurrence of at least 1 colorectal polyp, and the secondary outcomes were the occurrence of advanced lesions and adenoma multiplicity. Two previous animal studies coupled with the epidemiological evidence suggested that the results of this randomized controlled trial would be positive. Unfortunately, the results were not positive. It was concluded from this trial that folic acid did not reduce colorectal adenoma risk. In fact, there was a drift in the outcome data from the trial that associated folic acid supplementation at this level with higher risks of multiple

adenomas in people who had the most high-risk backgrounds.⁵

The publication of this trial follows closely on the heels of the publication in 2006 of another randomized, placebo-controlled trial of folic acid and other B vitamins in the prevention of vascular disease in people with elevated serum homocysteine.⁶ That trial failed to demonstrate a role of supplemental folic acid and other B vitamins in the prevention of vascular disease. The questions that follow the results of these studies are “Why the unexpected outcomes?” and “What does this mean with regard to the safety of folic acid supplementation?”

FOLATES AND PHYSIOLOGY

To address these questions it is important to review what has been learned about the source and role of folic acid and other folates in human physiology. Folates are found in numerous foods of animal and plant origin but are especially plentiful in dark green leafy vegetables (eg, spinach, lettuce, cabbage). Folates are very sensitive to heat, oxygen, and light, and therefore a considerable amount is lost during storage, processing, and food preparation. In a mixed diet, only a small amount of the total folates are present in the most bioavailable form of monoglutamates, the larger portion present as polyglutamates that have to be transformed by intestinal enzymes into the more available monoglutamate form. These enzymes are highly polymorphic, and some of the variants result in less efficient conversion of food folates to the monoglutamate form. The common form of supplementary folic acid is the monoglutamate form, so in this case folic acid administered as a food supplement has a higher bioavailability than food folates.

Once absorbed, folic acid monoglutamates are reduced and methylated in the intestinal mucosa to tetrahydrofolates. Tetrahydrofolate then serves as an intermediate acceptor and donor of methyl, methylene, methenyl, formyl, and formimino residues in intermediary metabolism. 5,10-methylene-tetrahydrofolate and 10-formyl-tetrahydrofolate are involved in nucleic acid synthesis for the formation of DNA and RNA. The conversion of 5,10-methylene-tetrahydrofolate to 5-methyl-tetrahydrofolate by the enzyme methylene-tetrahydrofolate reductase (MTHFR) allows for the formation of S-adenosylmethionine (SAM) through the remethylation of homocysteine. It is important to note that there are many metabolic steps involved in these conversions, each requiring its own specific enzyme, many of which are dependent upon specific B vitamin-derived cofactors such as riboflavin for

FAD (flavin adenine dinucleotide), vitamin B₁₂, vitamin B₆, and betaine (trimethylglycine). The formation of SAM is a critical event in cellular metabolism in that it is the source of methyl groups for all cellular processes that involve methylation, including the formation of neurotransmitters, phosphatidylcholine for membrane and myelin composition, methylation of endogenous estrogen metabolites and other detoxification/biotransformation reactions, and posttranslational methylation of arginine-rich proteins, including histones that make up the composition of the nucleosome. SAM is also the source of methyl groups that are involved in the epigenetic modulation of the genome through the methylation of CpG islands within the promoter regions of various genes.⁷ The emerging understanding of the role that folic acid plays in epigenetic changes in gene expression may help us to understand why the clinical intervention trials on folic acid have not resulted in positive outcomes.

It is now recognized that our genes are not “hardwired” to produce a pre-orchestrated outcome in the phenotype. Rather, our genes are pluripotential and demonstrate many options to be expressed in the phenotype depending upon regulatory elements that reside within the epigenome. Whereas the genome is fixed once the sperm has united with the egg, the epigenome is modifiable on the basis of what a person has been exposed to.

The control of how the genes will be expressed resides within the promoter regions of the genome that are under epigenomic regulation through processes such as methylation, phosphorylation by kinase enzymes, ubiquitination, and acetylation.⁸ Each of these processes is influenced by diet and environmental exposures.

Methylation of the genome that includes both the promoter regions of the chromosomes and proteins that make up the nucleosome is in part dependent on the availability of SAM, which in turn is dependent on the amount of 5-methyl-tetrahydrofolate and ultimately on the availability of folic acid. It is well known that one common genetic uniqueness in the availability of SAM is the TT polymorphism of the enzyme MTHFR. This single nucleotide polymorphism (SNP) is present in approximately 10% to 15% of the population and results in a reduced conversion of folic acid to 5-methyl-THF and ultimately to SAM.⁹ The presence of this polymorphism for folate metabolism results in differing responses to folate in the diet.

Methylation is therefore a reversible modification of DNA participating in epigenetic regulation of gene expression. It has become clear that both atherosclerosis and colorectal cancer are associated with aberrant DNA methylation patterns at the promoter regions of genes that control cellular proliferation and malignancy. It has recently been shown that during both early atherosclerosis and colon cell oncogenesis alterations in DNA methylation occur and nutritional and other factors beyond that of folate effects on homocysteine contribute to the regulation of these processes.¹⁰

WHAT DOES THIS TELL US?

These recent discoveries shed light on the absence of positive

clinical outcomes from the controlled intervention trials on the use of folic acid for the prevention of colorectal adenomas and vascular disease. It has been found that inflammatory processes play an important role in modulating epigenetic methylation patterns beyond that of the role of SAM and folic acid in this process.¹¹ Methylation of the promoter region of oncogenes results in their “silencing”; therefore, hypomethylation of the promoter regions can result in increased sensitivity to carcinogens and the tumor initiation process. On the other hand, hypermethylation of the promoter regions of tumor suppressor genes can result in silencing of the expression of these important proteins that reduce the risk of oncogenesis. Inflammation has been associated with the alteration of epigenetic methylation patterns where there are hypomethylated promoter regions of protooncogenes and hypermethylation of promoter regions of tumor suppressor genes.¹²

The process of regulation of the methylation patterns of the epigenome is therefore much more than just providing a specific amount of folic acid in the diet. Chronic inflammation, the role of other nutrients on SAM production and availability and the presence of various polymorphisms are a few of the specific variables that can modify the risk to chronic diseases associated with altered methylation patterns.¹⁴ In colorectal cancers the MTHFR polymorphism appears to play a role in determining the sufficiency of the folate cellular pool to properly regulate genomic methylation.¹⁵

So what can we conclude regarding folate supplementation for the prevention of chronic disease? It has been demonstrated that folic acid supplementation will improve methylation patterns in people with hypomethylated regions of the genome.¹⁵ It has also been shown that hypomethylation of the promoter regions of some genes is associated with the hypermethylation of the promoter regions of other genes and that folic acid supplementation can assist in normalizing these methylation patterns.¹⁶ It has also recently been demonstrated that folic acid and vitamin B₁₂ supplementation (5 mg folate and 1.25 mg B₁₂ daily) in people with previous colorectal adenomas did not favorably influence uracil incorporation and promoter gene methylation in rectal mucosal DNA.¹⁷ The authors of this later study indicate that the results might be reflective of the fact that the intervention trials are not using the complex set of nutrients that are necessary to properly regulate methylation patterns in people with increased risk. It may be that what we are learning from the various vitamin intervention trials is that the methodology for these trials needs to stratify the participants for specific common polymorphisms that display enhanced sensitivity to the nutrient(s) to be evaluated. It is also possible that clinical trials with various nutrients, as contrasted to drug trials of a single molecule against a single endpoint, need to be designed with a mixture of nutrients that more realistically reflects how nutrition impacts the physiological network.

Taken as a whole, these studies on folate suggest the following:

1. Homocysteine is a partial surrogate marker for methylation capacity related to epigenetic modifications of the genome.

2. Folic acid is only one of many nutrients that are necessary to regulate methylation.
3. Inflammation plays a role in directing epigenetic methylation beyond that of folic acid status.
4. Many genetic polymorphisms play a role in the methylation, homocysteine, and gene-silencing story that can modify the need for specific nutrients for optimal phenotypic regulation.
5. Folic acid supplements in individuals with marginal folate status for the prevention of dysplasias seems justified across numerous chronic conditions, but higher folate supplements in people with existing carcinomas should be approached carefully.
6. The folate story in prevention of chronic diseases is still evolving, but it can be said that folate is not part of just a metabolic pathway but rather a systems biology network with multiple impacts on the physiological web.

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