Bringing Individuality to Public Health Recommendations\textsuperscript{1,2}

Patrick J. Stover\textsuperscript{3} and Cutberto Garza
Division of Nutritional Sciences, Cornell University, Ithaca, NY 14853

ABSTRACT The data generated from the human genome project offers unprecedented opportunities to elucidate the etiology of chronic diseases and developmental anomalies that arise from deleterious genome-diet interactions. Folate metabolism is an attractive system to explore such relationships. Folate is necessary for the synthesis of purine and thymidine deoxyribonucleotides and S-adenosylmethionine, a cofactor required for DNA methylation. Impaired folate metabolism results from primary folate deficiency, alcohol, gastrointestinal disorders that result in malabsorption, single nucleotide polymorphisms, increased folate catabolism and secondary nutrient deficiencies in vitamin B-6, vitamin B-12 and iron arising from a variety of pathologies. Any of these conditions singly or in combination influence DNA synthesis, DNA integrity, allelic-specific gene expression, chromatine structure and DNA mutation rates. Biochemical manifestations of impaired folate metabolism include increased uracil uptake into DNA, altered DNA methylation status and elevated homocysteine and S-adenosylhomocysteine in serum and tissues. These biochemical changes are associated with risk for cancer, cardiovascular disease, neural tube defects and some neuropsychopathies and anemia, although direct causative mechanisms have not been established in all cases. Interactions between folate and the genome are reciprocal; polymorphisms in key genes influence folate nutritional requirements, indicating that dietary folate adequacy likely exerts selective pressure and thereby influences genetic variation. Other studies indicate that exposure to excess folate, perhaps at levels that occur at the upper end of the intake distribution curve, may have unintended consequences in promoting embryo viability. Therefore individualizing folic acid dietary recommendations necessitates a detailed understanding of all genetic and physiological variables that influence the interaction of folate with the genome and their relationship to the disease process. J. Nutr. 132: 2476S-2480S, 2002.

KEY WORDS: folate \textbullet} methyltransferase \textbullet} diet \textbullet} public health

The human genome project has ushered in unprecedented opportunities for research and discovery in the nutritional sciences. These opportunities are complemented by recent progress in the social and biological sciences, most notably in genetics, genomic technologies and the behavioral sciences. The etiologies of most chronic diseases have interactive nutritional and heritable components. Elucidating the mechanisms that underlie these interactions requires the integration of knowledge and research methodologies from both nutrition and genetics. The completion of the human genome project provides the full inventory of genes that specify all of the components that constitute human cells (1), thereby permitting the identification of the composite sets of genes that modulate metabolic pathways and nutrient requirements. Equally important, ongoing initiatives to identify and map single nucleotide polymorphisms (SNP)\textsuperscript{4} within the human genome offer the possibility of understanding the molecular basis for genetic-environmental determinants of human phenotypic variation, especially as related to diet and disease (2,3). Interpretation of genetic maps, with insight derived from numerous biochemical and nutritional functional data, should lead to a more complete understanding of the etiology of complex chronic diseases, a possibility that hereto seemed unimaginable. The benefits of understanding the interrelationships among genetic variation, nutrition and age are enormous, for they represent the fundamental goal of the nutritional sciences: to tailor nutritional requirements to the

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\textsuperscript{3} To whom correspondence should be addressed.

E-mail: PJS13@cornell.edu.

\textsuperscript{4} Abbreviations used: \textit{Axd}, axial defects mutation; \textit{C677T}, cytosine-to-thymine transition at position 677; \textit{MTHFR}, methylenetetrahydrofolate reductase; \textit{NDT}, neural tube defects; \textit{Pax3}, paired box gene 3; \textit{SAH}, S-adenosylhomocysteine; \textit{SAM}, S-adenosylmethionine; SNP, single nucleotide polymorphism.

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individual and thereby optimize diets for health maintenance, disease prevention and disease management.

There are many obstacles to achieving individualized dietary requirements, both in the comprehensive identification of the genetic and environmental risk factors and in the design of effective preventative and therapeutic regimens that optimize outcomes. Although the generation of SNP databases represents an unparalleled achievement, its significance will remain unrealized without successful efforts to develop methods and strategies for effectively mining these resources. Recently, Schork et al. (2,3) reviewed the challenges of ascribing the contribution of any individual SNP or haplotype to complex phenotypes. Current statistical methods for analyzing genetic databases are limited by several factors: 1) most genetic variation is phenotypically silent or undetectable because of our limited knowledge or analytic detection capabilities; 2) genetically induced phenotype can be modified by epigenetic "overlays"; 3) the risk for chronic disease results from deleterious interactive combinations of genes (including allelic variants: SNP, haplotypes) and environmental variables. The challenges related to identification of risk factors can be mitigated, and the problems can be focused by insights into gene function, gene-nutrient interactions and/or metabolic and physiological regulation of the impacted systems. Once key genetic and environmental risk factors are identified, rational studies (23) become essential to understand the biological mechanisms responsible for the functional outcomes of interest. Only after this information is available can human metabolic studies and intervention trials be undertaken to develop optimized nutrient therapies and interventions for short term and perhaps long term disease prevention and management.

Folate-dependent one-carbon metabolism represents an ideal system to explore the relationships among nutrition, genetic variation and disease. Folate metabolism is necessary for the synthesis and structural integrity of DNA, and the methyl groups it provides regulate gene expression, chromatin structure and thus epigenetic regulation. The relationship between folate metabolism and the genome is reciprocal; studies have demonstrated that optimal dietary folate requirements are influenced by genetic variation and disease (4). Folate metabolism is influenced by secondary nutrient deficiencies as well and thereby serves as a more general conduit whereby nutrition can influence genome function and stability (5). Finally, impairments in folate metabolism are strongly associated with several seemingly unrelated complex diseases and developmental anomalies (6,7). This article summarizes current knowledge related to the interaction of folate metabolism and the human genome and discusses current limitations to individualizing public health recommendations for folate.

DISCUSSION

Folate metabolism and biochemical indices of impaired folate metabolism

Folate cofactors are a family of structurally related compounds that carry one-carbon units at three different oxidation states (8). Folate polyglutamates, the active cofactor forms of the vitamin, donate or accept one-carbon units in a set of reactions characterized as one-carbon metabolism, which occurs in the mitochondria and in the cytoplasm (5,8). One-carbon metabolism in the cytoplasm is necessary for the de novo synthesis of purines and thymidylate and for the remethylation of homocysteine to methionine. Methionine can be converted to form S-adenosylmethionine (SAM), a cofactor and one-carbon donor for numerous other methylation reactions including the methylation of DNA and histone proteins (9). It is now accepted that mitochondrial one-carbon metabolism generates glycine and formate from cytoplasmically derived serine, the primary source of folate-derived one-carbon units (10). Mitochondria-derived formate traverses to the cytoplasm where it is a primary source of one-carbon units for cytoplasmic one-carbon metabolism.

Disruptions of folate metabolism are highly correlated with human disease and congenital anomalies including epithelial cancers, peripheral neuropathies, cardiovascular disease and neural tube defects (NTD) (7,11–16). Impaired folate metabolism results from primary folate deficiency, gastrointestinal disorders that result in malabsorption, single nucleotide polymorphisms, increased folate catabolism and secondary nutrient deficiencies in vitamin B-6, vitamin B-12 and iron arising from a variety of pathologies (17–20). In addition, certain dietary factors can interfere with folate bioavailability (21). Whole body folate status is regulated by folate intake, intestinal absorption and catabolism (22). At the cellular level, little is known about the mechanisms that regulate intracellular folate concentrations. There is evidence that cellular folate concentrations are tightly regulated by folate degradation, and animal studies have demonstrated that increased rates of folate catabolism occur during states of rapid cell proliferation including cancer and pregnancy (22). Interestingly, cells are highly susceptible to folate deficiency during states of increased folate turnover, because they do not accumulate excess folate relative to their binding capacity (22). Therefore it is apparent that physiological mechanisms exist that can trigger localized functional folate deficiency in the absence of a "generalized" dietary deficiency.

Folate metabolism also can be disrupted in the absence of overt folate deficiency by other primary or secondary nutrient deficiencies and/or polymorphisms in key folate enzymes. Several SNP have been identified in genes that encode folate-dependent enzymes (23). The resulting amino acid substitutions influence protein stability and/or the catalytic properties of the enzymes in vitro and impact metabolism in vivo. Vitamin B-6 or B-12 deficiency can impair folate metabolism because these vitamins also serve as cofactors for folate-dependent enzymes (5). Other dietary and hormonal factors including iron are known to alter the expression of genes that encode folate-dependent enzymes and thereby influence metabolic fluxes among the various folate-dependent anabolic pathways (17,18). Therefore other primary or secondary nutrient deficiencies can cause symptomatic conditioned folate deficiency without evidence for systemic primary folate deficiency. The associated biochemical mechanisms accounting for these effects are not firmly established. However, elucidating the biochemical mechanisms that regulate intracellular folate concentrations and metabolic fluxes is critical for an understanding of the complex relationships among folate status, folate metabolism and disease and to design targeted nutrient therapies.

Disruption of folate-requiring anabolic pathways leads to identifiable biochemical abnormalities. Homocysteine remethylation and thymidylate biosynthesis are among the most vulnerable pathways (24–26). Elevated homocysteine and/or S-adenosylhomocysteine (SAH) in serum and tissue are established biomarkers for impaired folate metabolism (24,27). Urinary excretion of formiminoglutamate, increased uracil content in DNA and alterations in cellular morphology including hyposegmentation of neutrophils also result from impaired folate metabolism (17). The relationships between altered folate metabolism and the appearance of these biomarkers are
supported and predicted by established biochemical mechanisms and metabolic pathways. However, the relationship between folate and disease and the roles, if any, of these biomarkers in disease processes are virtually unknown. Several studies have suggested that the chemical properties of the sulfur amino acid homocysteine account for the folate-disease relationship (28). Other studies indicate that SAH accumulation resulting from elevated homocysteine influences the activity of certain SAM-dependent methyltransferases (9). SAH is an effective inhibitor of some SAM-dependent methylases, indicating that certain methylation reactions respond to or are regulated by the efficiency of the homocysteine remethylation pathway and can be disrupted by impairments in folate metabolism in general (9). These SAH-sensitive reactions include the methylation of DNA, RNA and proteins and the synthesis and degradation of neurotransmitters and catecholamines (9).

Impairments in thymidylate biosynthesis and homocysteine remethylation can impact directly genome structure and stability (14–16). Studies of humans and animals have shown an inverse association between folate status and uracil content in DNA, presumably resulting from impaired thymidylate synthesis and subsequent misincorporation of dUTP into DNA (26). Cancerous cells, which are often folate deficient, contain increased uracil in DNA (16). Folate deficiency also results in increased frequency of chromosomal strand breaks, and folate-deficient cells in culture are more susceptible to the mutagenic effects of radiation and alkylating agents than those in folate-sufficient medium (16). Elevations in homocysteine also influence the activity of DNA (cytosine-5)-methyltransferase (EC 2.1.1.37) (9). Folate status parallels DNA methylation density in lymphocyte DNA (29), and folate deficiency in mice results in decreased hepatic genome-wide methylation density in some but not all studies (15).

Altered folate biochemistry and disease

Population studies have revealed associations between folate status and risk for certain cancers, NTD and cardiovascular disease (12,15,16,23,30–35). It is well established that folic acid prevents the occurrence and recurrence of NTD, although the biochemical and developmental mechanisms whereby this protection is afforded are unknown. In theory, one would predict that folate-responsive NTD arise due to specific metabolic disruptions, including impaired synthesis of DNA nucleotide precursors or downstream effects resulting from impaired methionine (or SAM) synthesis, including alterations in DNA methylation. Elevated serum homocysteine and polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) (EC 1.5.1.20) and methionine synthase (5-methyltetrahydrofolate-homocysteine 5-methyltransferase; EC 2.1.1.13) gene that inhibit methionine synthesis are correlated with risk for human NTD, indicating that NTD result from impairments in the homocysteine remethylation pathway or in other affected pathways for which homocysteine serves as a marker. However, this notion is not fully supported by animal studies. Maternal folate supplementation is protective in some mouse models of NTD and, for some mutations, maternal folate supplementation can be replaced with metabolic products of folate metabolism. Thymidine, but not methionine, can replace folate in preventing NTD in the paired box gene 3 (Pax3) mouse model (36), whereas methionine alone is protective for axial defects (Axl) mutant mice (37). These results are not necessarily contradictory with respect to the ultimate biochemical mechanism. There is accumulating evidence that intracellular folate concentrations are limiting, therefore indicating that the various metabolic pathways compete for a limited pool of folate cofactors. Disruption or enhancement of any given pathway may impact flux through another pathway. For example, supplementation of Pax3 mice with thymidine may permit increased flux of one-carbon units through the homocysteine pathway. Therefore definitive metabolic mechanism(s) that account for the folate-NTD association remain elusive.

Current folate fortification policy in the United States seeks to lower the occurrence and recurrence of NTD, and it is widely accepted that recurrent NTD are associated with specific genetically distinct subpopulations. It is apparent that folate can rescue undesired developmental outcomes that have a genetic etiology. At the time that maternal folic acid supplementation was recommended, it was assumed that the genotypes associated with folate-responsive NTD risk would be isolated to specific and identifiable alleles, presumably within genes that encode folate-dependent enzymes, and that these mutations would influence the structure, function and activity of encoded proteins. These hypotheses were corroborated as defined polymorphisms in genes that encode folate-dependent enzymes were demonstrated to be associated with high risk for NTD (23,38). However, there are other experimental animal data that indicate that increased folate intake during pregnancy may have more general effects on rescuing developmental processes associated with genetic mutations including severe genetic deletions. In mouse animal models, maternal supplementation with folic acid during gestation can ameliorate NTD that result from severe genetic disruptions that are seemingly unrelated to folate metabolism. Maternal supplementation with folic acid at high levels can prevent NTD resulting from deletion of Pax3 (36), the cartilage homoprotein 1 (Cart1) homeobox gene (39) and Crooked tail (40), whereas methionine, but not folic acid, can alter the frequency of NTD in Axl mutants (37). Folate supplementation also decreases NTD incidence resulting from maternal hyperthermia in mice (41). Folate can be a limiting nutrient for cell growth in vitro; therefore maternal folate supplementation may rescue partially any number of genetic defects or environmental insults that result from decreased rates of cell division. Alternatively, the folate-NTD relationship may be due to deleterious folate-genome interactions. DNA methylation may influence the expression of 10% of mammalian genes (42), indicating that impaired folate metabolism may influence the expression of many genes that are required for neural tube formation. Therefore the genetic and nutritional interactions associated with NTD risk are likely to be complex, and, as a minimum, the contribution of the salient genetic and nutritional variations must be identified before etiology is established and future recommendations put forward.

Epidemiological studies have indicated an inverse relationship between folate status and risk for cancer of the colon (15,16,31). Risk for colon cancer has both a genetic and a nutritional etiology. Humans with a common polymorphism in the MTHFR gene have a much lower specific activity of MTHFR, which results in impaired 5-methyltetrahydrofolate synthesis and a reduced capacity to remethylate homocysteine. Homozygotes for this polymorphism have decreased incidence of colorectal cancer compared with heterozygous or normal genotypes in the absence of folate deficiency (31). Potential biochemical mechanisms for the protective effects of folate and colon cancer include decreased uracil content in DNA and folate-dependent changes in DNA methylation density, which leads to downstream effects on methylation-sensitive gene expression.
Folate nutrition compensating for genetic defects

Since the initiation of folate fortification and maternal folate supplementation during pregnancy, there is evidence that the number of reported spontaneous abortions is increasing (43–45). The authors of these studies suggest that elevated maternal folate may prolong pregnancies that would otherwise miscarry very early in pregnancy. Other studies have indicated that human maternal supplementation with folic acid may rescue embryos that otherwise would undergo spontaneous abortion or escape implantation. MTHFR polymorphisms, singly or in combination with other MTHFR polymorphisms, are risk factors for decreased fetal viability and recurrent embryo loss in early pregnancy (46–51). Interestingly, one study implied that the cytosine-to-thymine transition at position 677 (C677T) of the MTHFR gene polymorphism is not in Hardy-Weinberg equilibrium, given that homozygotes (thymine/thymine dinucleotide) were underrepresented in a population (52). This study, although controversial, presents evidence that this MTHFR polymorphism has become more prevalent as a result of maternal folate supplementation. Although the results of this study are equivocal, both the human and murine data indicate that high levels of maternal folate may rescue genotypes that otherwise would be deleterious or lethal. These studies and results from the aforementioned animal studies that demonstrate a protective effect of folate against severe genetic lesions indicate that high levels of maternal folate during gestation may have adverse unintended consequences. Well-designed animal studies that investigate the effects of maternal folate on fetal viability, and perhaps more subtle effects on fetal physiology and disease susceptibility, are clearly warranted. Results from these studies may lead to better targeted nutritional interventions that prevent folate-responsive NTD while avoiding potential adverse outcomes.

Future considerations

The etiology of late onset chronic disease has long been recognized as multifactorial, with poor nutrition and identifyable genetic factors contributing to risk. However, there is an emerging awareness that developmentally determined epigenetic factors also contribute to disease risk in adulthood (53–56). The notion that maternal and fetal environments contribute to susceptibility to late onset disease, the fetal origins hypothesis, has focused renewed attention on the effects of maternal nutrition during the various stages of fetal development on outcomes that do not become apparent until later in life. Fetal adaptations to maternal nutritional status, recently reviewed and referred to as metabolic imprints, are believed to occur within critical developmental windows that include organogenesis and central nervous system development (53). Potential epigenetic mechanisms accounting for the permanent adaptation associated with metabolic imprints are not established but likely involve metabolic or nutrient-induced alternations of genome structure, gene expression or gene mutation. The adaptation, once permanently established in a single cell, can expand and colonize the entire organ or organ system if the alteration imparts a selective growth advantage to that particular imprinted genotype (53). Potential mechanisms for these imprints include changes in DNA methylation patterns and DNA mutation rates, factors that are influenced by folate status. The potential exists that maternal folate status can induce or permit genomic alternations that maximize embryonic and fetal survival while simultaneously imparting subtle yet lasting effects on disease risk throughout the life cycle. In summary, intervention studies have shown that nutrition is a safe and effective means to modify or manipulate genome structure, function, expression and stability for benefit. However, our understanding of the role of nutrient-mediated genome modulation in altering disease susceptibility, initiation and progression is only in its infancy; therefore current dietary approaches lack predictive value. Clearly, the potential exists to individualize folate requirements to maternal/fetal genotypes and thereby optimize birth outcomes, but this task necessitates a comprehensive understanding of the underlying genetic, nutritional and biochemical etiologies of these diseases. The rational design of effective interventions and therapies that lead to the development of genomically based, tailor-made diets for disease prevention and management necessitates the integration of at least the most relevant statistical, biological and genomic approaches to address the interaction of risk factors. However, as we increase our understanding of the role nutrition plays in initiating, accelerating or preventing disease processes on different genetic backgrounds, the potential for scientifically based dietary guidelines that optimize human health seems achievable.

LITERATURE CITED


