Published OnlineFirst July 13, 2011; DOI:10.1158/1055-9965.EPI-10-1352

Cancer Epidemiology, Biomarkers & Prevention

Mathematical Modeling Predicts the Effect of Folate Deficiency and Excess on Cancer-Related Biomarkers

Marian L. Neuhouser, H. Frederik Nijhout, Jesse F. Gregory III, et al.

Cancer Epidemiol Biomarkers Prev 2011;20:1912-1917. Published OnlineFirst July 13, 2011.

Published OnlineFirst July 13, 2011; DOI:10.1158/1055-9965.EPI-10-1352

Cancer Epidemiology, Biomarkers & Prevention

Research Article

Mathematical Modeling Predicts the Effect of Folate Deficiency and Excess on Cancer-Related Biomarkers

Marian L. Neuhouser[1], H. Frederik Nijhout[2], Jesse F. Gregory III[4], Michael C. Reed[3], S. Jill James[5], Amy Liu[1], Barry Shane[6], and Cornelia M. Ulrich[1,7]

Abstract

Background: Folate is an essential B-vitamin that mediates one-carbon metabolism reactions, including nucleotide synthesis and others related to carcinogenesis. Both low- and high-folate status influences carcinogenesis.

Methods: We used a mathematical model of folate-mediated one-carbon metabolism to predict the effect of a range of intracellular epithelial folate concentrations (0.25–15.0 μmol/L) on methylation rate and purine and thymidylate synthesis. We also examined the interaction of these folate concentrations with polymorphisms in two enzymes [methylene tetrahydrofolate reductase (MTHFR) and thymidylate synthase (TS)] in relation to the biochemical products.

Results: TS enzyme reaction rate increased markedly in response to the modeled higher intracellular folate concentrations. Changes in methylation rate were modest, whereas purine synthesis was only minimally related to increases in folate concentrations with an apparent threshold effect at 5.0 to 6.0 μmol/L. The relationship between folate concentrations and thymidylate synthesis was modified by genetic variation in TS but less so by variation in MTHFR. These gene–folate interactions modestly influenced purine synthesis in a nonlinear manner but only affected methylation rate under conditions of very high MTHFR activity.

Conclusion: Thymidylate synthesis is very sensitive to changes in epithelial intracellular folate and increased nearly fivefold under conditions of high intracellular folate. Individuals with genetic variations causing reduced TS activity may present even greater susceptibility to excessive folate.

Impact: Our observation that thymidylate synthesis increases dramatically under conditions of very elevated intracellular folate provides biological support to observations that excessive folic acid intake increases risk of both precursor lesions (i.e., colorectal adenomas) and cancer. *Cancer Epidemiol Biomarkers Prev; 20(9); 1912–7. ©2011 AACR.*

Introduction

Folate is a water-soluble B-complex vitamin that is essential for human health (1). The primary function of folate is as a carrier of single-carbon units used in many important biochemical reactions, including those related to amino acid metabolism, nucleotide synthesis, and numerous methyl-transferase reactions, including DNA methylation (2). These biochemical pathways of folate-mediated one-carbon metabolism (FOCM) are complex, involving numerous enzymes, substrates, cofactors, and various degrees of oxidized or reduced folate (1, 3). Furthermore, the proteins controlling this pathway are encoded by genes in which polymorphic variants affecting enzyme activity and health outcomes have been identified (4, 5).

Understanding the metabolic functions of FOCM and their relationship to cancer risk is a topic of considerable importance. Folate deficiency has been associated with increased risk for cancer of the colon, breast, and pancreas (3, 6, 7). Conversely, high folic acid supplementation has been associated with increased risk of colorectal adenomas (8) and increased risk of breast cancer (9). Investigations of the health effects of high to excessive folic acid may be particularly important, given the high exposure of the U.S. population to folic acid through the common practice of high-dose dietary supplement use. In addition, many consumers eat other highly fortified products, such as cereals, nutrition bars, and fortified beverages (10). Together, these food and supplement

Authors' Affiliations: [1]Cancer Prevention Program, Fred Hutchinson Cancer Research Center, Seattle, Washington; Departments of [2]Biology and [3]Mathematics, Duke University, Durham, North Carolina; [4]Food Science & Human Nutrition Department, University of Florida, Gainesville, Florida; [5]Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, Arkansas; [6]Department of Nutritional Sciences & Toxicology, University of California, Berkeley, California; and [7]German Cancer Research Center and National Center for Tumor Diseases (NCT), Heidelberg, Germany

Corresponding Author: Marian L. Neuhouser, Cancer Prevention Program, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, M4-B402, Seattle, WA 98019. Phone: 206-667-4797; Fax: 206-667-7850; E-mail: mneuhous@fhcrc.org

doi: 10.1158/1055-9965.EPI-10-1352

Published OnlineFirst July 13, 2011; DOI:10.1158/1055-9965.EPI-10-1352

practices may place some consumers at risk of exceeding the tolerable upper limit intake of 1,000 μg folic acid per day, as specified by the Food & Nutrition Board of the Institute of Medicine (1). However, empirically testing low- and high-folate intake in human populations is not altogether satisfactory, either in terms of understanding the health risks or comprehending the biology. Furthermore, because there are some concerns about high-dose folic acid (8, 10–12), it is not ethical to carry out dose–response studies that may result in harm. One approach to understanding the potential effects of folic acid on metabolism is by mathematical modeling of folate biochemistry (13, 14). Our model allows us to simulate the effects of nutritional variation (e.g., in folate intake) on biomarkers related to carcinogenesis (e.g., methylation), the effects of known genetic mutations in FOCM enzymes, and gene–nutrient interactions (13, 14). In this article, our objective was to understand the effects of low- and high-folate concentrations, such as that which might be present in either a folate deficiency or folate excess and the subsequent relationship to numerous important processes of FOCM, such as methionine synthesis, purine synthesis, and thymidylate synthesis. We used a model of epithelial FOCM, consistent with the notion that some organs, such as the colon, may be the most susceptible to folate deficiency or excess.

Methods and Results

Overview of the model

Detailed methods describing our model of FOCM are published (13). Briefly, the model simulates the multiple, interconnected biochemical reactions of folate metabolism. The model was built by using known biochemistry and standard reaction kinetics; differential equations were used to describe each enzymatic reaction in the context of variable substrate availability. In addition, the model incorporated data on known regulatory mechanisms (e.g., substrate inhibition or long-range inhibition; ref. 15). Long-range interactions between the interconnected folate and methionine cycles, which regulate the properties of 1-carbon metabolism, were also included (14, 15). The model uses published data from various mammalian species and their tissues with respect to folate–enzyme kinetics and regulatory mechanisms.

For this article, our FOCM model was used to predict: (i) the effect of a broad range of intracellular folate concentrations simulating variation in folate status on mechanisms relevant to carcinogenesis (e.g., methylation rate, purine synthesis, and thymidylate synthesis); and (ii) interaction of functional polymorphisms in key FOCM enzymes (MTHFR and TS), with varying folate concentrations in relation to methylation rate and purine/thymidylate synthesis. These enzymes were chosen for their known regulatory function (MTHFR) and critical role in nucleotide synthesis (TS). A number of observational and intervention studies, some of which included folate supplementation, have reported total folate concentrations in human colonic cells ranging from 0.2 to 6.9 μmol/L, with an average around 1.0 μmol/L (16–21). One of these intervention studies reported 5-Me-THF concentrations (rather than total folate) of 1 to 2 μmol/L (21). On the basis of these findings, we modeled a physiologic range of folate concentrations that might be expected in the colonic epithelial cell.

An intracellular concentration of 1.0 μmol/L was chosen as the reference or standard value, wherein the methylation rate and purine/thymidylate synthesis were assumed to function at 100%. All other modeled values are relative to this reference concentration and enzyme activity.

Figure 1 models the rates of purine synthesis, thymidylate synthesis, and methylation rate across a range of intracellular folate values, relative to 1.0 μmol/L and the ensuing effect on purine synthesis, thymidylate synthesis, and methylation capacity. The top panel illustrates the enzymatic rates as predicted by the model, whereas the bottom panel is the normalized rate relative to 1.0 μmol/L folate. The increase in thymidylate synthesis from 0.25 to 15.0 μmol/L is nearly 4-fold (top panel). Enzyme activity rate in relation to purine synthesis is only modest, with an apparent threshold effect around 4.0 to 6.0 μmol/L, followed by a slight decline at what

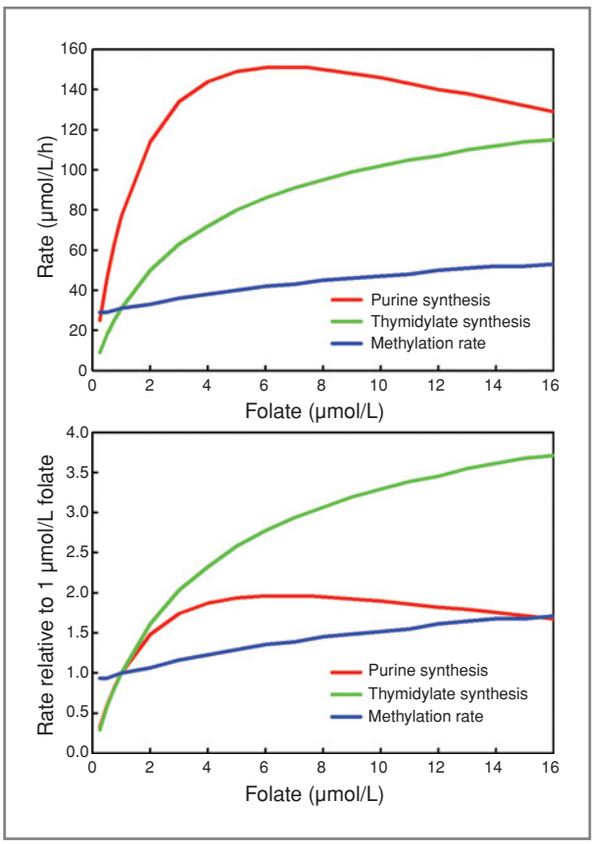

Figure 1. Purine and thymidylate synthesis and methylation rate.

Published OnlineFirst July 13, 2011; DOI:10.1158/1055-9965.EPI-10-1352

could be considered excessive intracellular concentrations (i.e., 15.0 μmol/L). Enzyme activity and subsequent increases in thymidylate synthesis change markedly in response to higher folate concentrations as evidenced by the steep slope in the bottom panel. The influence of increasing intracellular folate concentrations in epithelial cells on methylation capacity is characterized by a modest linear increase.

To further understand how variation in the intracellular folate concentrations may interact with genetic characteristics, we simultaneously modeled the range of folate concentrations (0.25 to 16.0 μmol/L), reflecting deficiency to excessive concentrations, jointly with variation in TS and MTHFR activity in relation to thymidylate synthesis, purine synthesis, and methylation rate (Figs. 2–4). MTHFR and TS were chosen because they have established common polymorphisms known to significantly affect gene expression or enzyme function. We have previously reported the influence of these variants independently on biomarker endpoints (22).

Figure 2 shows an interaction between TS activity and increasing folate concentrations in relation to thymidylate synthesis (top panel). This evidence suggests that individuals with genotypes affecting TS activity, gene expression, or mRNA stability may experience vastly different sensitivity in response to elevated folate concentrations. The greatest thymidylate synthesis occurs when both folate concentrations are high and thymidylate synthase activity is elevated. In the bottom panel of Figure 2, modest nonlinearity exists in the response for MTHFR, also suggesting an interaction, but one in which thymidylate synthesis is not nearly as high as in the top panel. In Figure 3, across different activity levels of TS or MTHFR, purine synthesis plateaus after folate concentrations reach approximately 5.0 to 7.0 μmol/L, although there may be some nonlinearity depending on the underlying enzyme activity. Figure 4 reflects the methylation rate, which shows a modest increase in methylation rate as folate increases. The effect of the enzyme activity is most pronounced under conditions of high-folate activity and high-TS or MTHFR activity in relation to methylation rate.

Discussion

This mathematical model of FOCM illustrates the effect of a range of epithelial intracellular folate concentrations

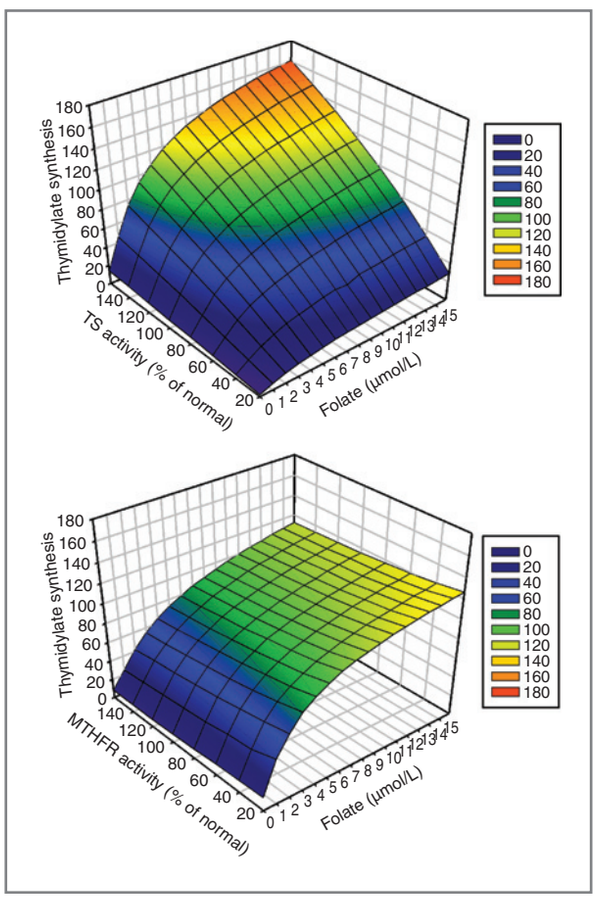

Figure 2. Thymidylate synthesis by folate concentrations, TS and MTHFR activity.

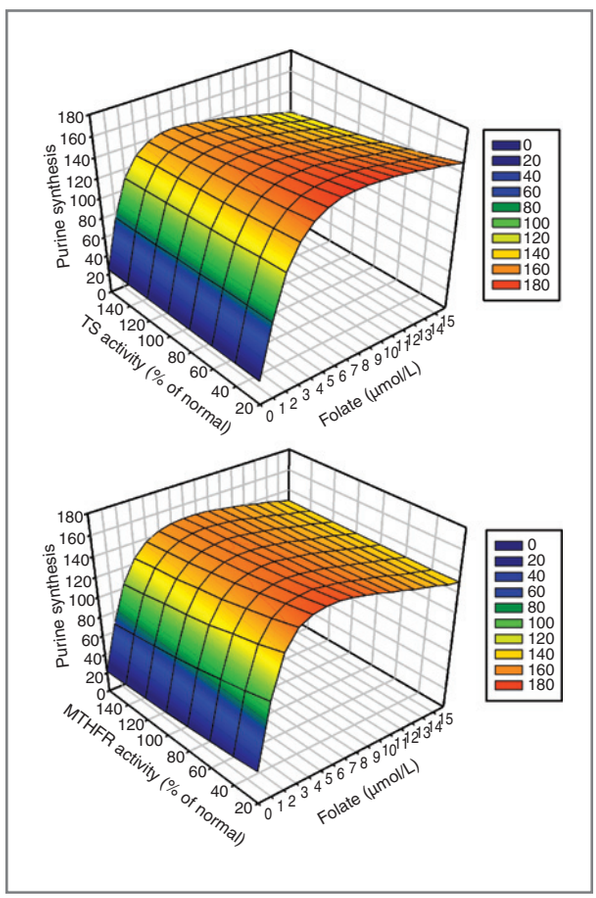

Figure 3. Purine synthesis by folate concentrations, TS and MTHFR activity.

Published OnlineFirst July 13, 2011; DOI:10.1158/1055-9965.EPI-10-1352

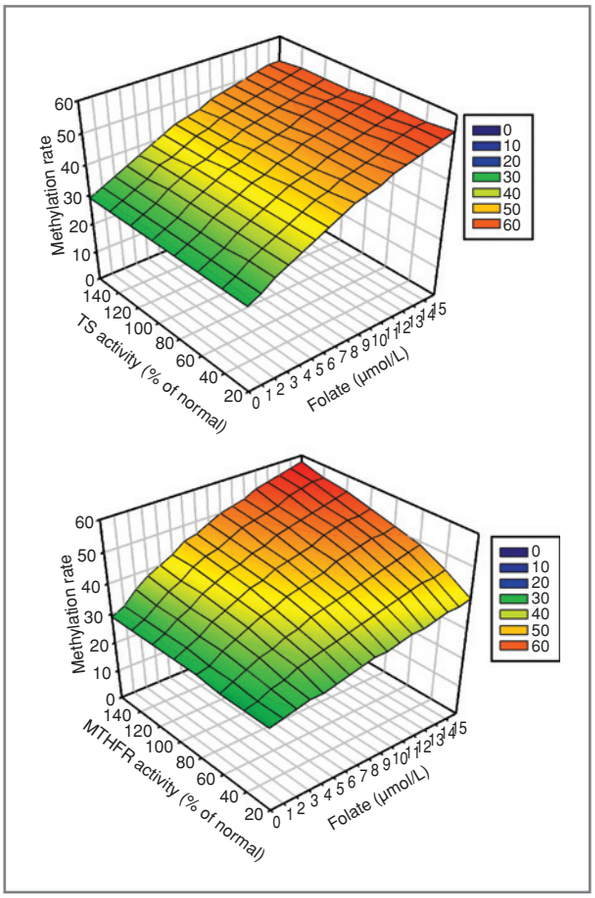

Figure 4. Methylation by folate concentrations, TS and MTHFR activity.

on 3 principal cancer-related biomarker outcomes: purine synthesis, thymidylate synthesis, and methylation rate. These results may be important because some American consumers are exposed to high intakes of folic acid through consumption of highly-fortified functional foods or dietary supplements. Some reports suggest that few exceed the Institute of Medicine's tolerable upper intake level of 1,000 μg folic acid per day (23–25) and that any excess intake does not seem to be attributable to consumption of enriched grains and cereals (23, 24). However, a recent NHANES report indicated that 2.6 million Americans aged 51 to 70 years exceed the tolerable upper intake level of 1,000 μg/d of folic acid (23). Thus, it seems that some, but not all, Americans consume excess folic acid and therefore understanding the biology of the extremes of intake is important. For example, a few observational studies and one randomized controlled trial have suggested that excessive folic acid intake, primarily from dietary supplements, may be detrimental to health (8, 9), but the biological mechanisms are not yet identified or well-understood. Thus, our goal was to understand the extent to which a range of high or low intracellular folate concentrations might influence biomarkers that relate to carcinogenesis.

Mathematical modeling is an important tool in the study of human systems biology. Modeling allows for the study of complex, nonlinear systems that are difficult to study in a purely experimental manner. Results from this approach can be used to formulate new hypotheses to test, where possible, *in vivo*. The principal finding from our mathematical modeling is that thymidylate synthesis is sensitive to changes in intracellular folate concentrations. Our model predicts that at normal physiologic folate concentrations (i.e., 1.0 μmol/L) thymidylate synthase activity is 30.0 μmol/h, whereas at supraphysiologic concentrations (i.e., 15.0 μmol/L) thymidylate synthase activity is increased to 118 μmol/h, corresponding to a nearly 4-fold increased synthesis of thymidylate. These simulations suggest that thymidylate synthesis activity may be driven in large part by intracellular folate concentrations. This is an important observation because some of the hypothesized adverse effects of high folate intake, particularly from synthetic sources, may increase intracellular folate to these supraphysiologic concentrations. However, we recognize that intracellular concentrations are influenced by transporters and other mechanisms that are not able to be modeled here. Nonetheless, these findings lend support to the observations that excessive folate intake may increase risk of colorectal adenoma as well as certain cancers, quite possibly due in part to increased thymidylate synthesis. Another consideration is that thymidylate synthase is the primary target for 5-fluorouracil, a chemotherapeutic drug used widely in cancer treatment, particularly colorectal cancer. The increased expression of thymidylate synthase has been associated with reduced survival and other adverse outcomes in colorectal cancer (26, 27).

Of interest is also the suggested interaction between both TS or MTHFR activity and increasing folate concentrations in affecting thymidylate synthesis. Our model predicts that genetic susceptibility, particularly the promoter polymorphisms in the *TS* gene, could have a major influence on the intracellular response to increasing folate: individuals with higher TS activity to begin with would be much more responsive to an increase in folate, as evidenced by the observed steep slope in thymidylate synthesis.

Our model predicts that purine synthesis will plateau with increasing concentrations of intracellular folate. On the basis of our genetic epidemiologic data, we have previously suggested that purine synthesis may be one of the key pathways supporting carcinogenesis (28); however, our model suggests that this would apply largely to the range from 1.0 to 5.0 μmol/L.

Finally, as we showed previously (15), methylation rate was relatively robust against changes in folate. What was most interesting was the robustness at the lower folate concentrations. Our model has previously shown that this stability is due to several regulatory mechanisms in the FOCM (14), which may be an indication of selective adaptation because methylation is central to many metabolic conversions and crucial for survival. This aspect of

Published OnlineFirst July 13, 2011; DOI:10.1158/1055-9965.EPI-10-1352

the model is not in complete agreement with other published data. In 2002, Friso and colleagues examined *MTHFR* genotype, plasma folate, and DNA methylation in 292 Italian study subjects. Those with low plasma folate and the *MTHFR T/T* genotype had significantly lower DNA methylation, suggesting a strong interaction of the genotype with folate status in relation to methylation (29). Some difference between our model results and those of Friso and colleagues might be because of the model's use of intracellular folate versus plasma folate, and the model predicts methylation rate not absolute status. Further *in vivo* research should be conducted to understand the relationship between *MTHFR* genotype, intracellular folate, and the methylation rate. Whereas some model output may not be completely intuitive or expected, the results still provide important questions for future research.

This study has several strengths. This mathematical model of FOCM has previously been shown to provide insights into folate metabolism, nutrient–gene interactions, and gene–gene interactions (22). The model has been tested extensively by using kinetic data from experimental studies and has done well for understanding other aspects of the folate pathway (13–15). One of the key strengths of the model is that it substitutes for unethical exposure of humans to either deficient or overly excessive intakes of folic acid for lengthy periods. Although depletion–repletion studies in humans have been extremely useful for establishing human folate requirements (1, 30), these metabolic studies do not test enzyme activity or synthesis of critical downstream molecules. Our simulations as presented in this article suggest that thymidylate synthesis, and to a lesser extent purine synthesis, are the most sensitive to changes in folate concentrations. Because thymidylate production is a critical step in DNA synthesis, this may help explain why high-dose folic acid supplements may lead to increased risk of colorectal adenomas, which require a steady flux of nucleotides for DNA synthesis and proliferation. There are also limitations. First, the range of intracellular folate concentrations (0.25–15.0 μmol/L) used for the simulations was hypothetical; true variation in actual intracellular folate concentrations as a function of dietary excess or deficiency may in fact differ from that used in the modeling. Furthermore, the intracellular concentrations are dependent upon transporters and folylpolyglutamate synthase (FPGS) levels, but sufficient data are not available to include either transporters or FPGS in the models. Nonetheless, the ranges provided give a useful illustration of the intended effect. Another limitation is that we are unable to differentiate between intakes of natural folate and synthetic folic acid or whether the source of intake would differentially affect intracellular folate concentrations. Some studies have suggested that the potentially detrimental consequence of synthetic folic acid is large amounts of unmetabolized folic acid in the circulation (31). In future studies, as our model expands, we hope to simulate the effect of synthetic folic acid.

In conclusion, our mathematical model of FOCM suggests that thymidylate synthesis increases nearly 5-fold under conditions of excess intracellular folate concentrations. Genetically predisposed individuals may experience even larger increases. Our model provides a possible link between the biochemical consequences resulting from high folic acid intake and potential increased risk of preneoplastic lesions or cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

Supported by the National Cancer Institute, United States Department of Health and Human Services, NCI R01 CA105437.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received December 30, 2010; revised June 9, 2011; accepted July 5, 2011; published OnlineFirst July 13, 2011.

References

1. Panel on Folate, other B Vitamins and Choline; Institute of Medicine. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, B12, Pantothenic Acid, Biotin and Choline. Washington DC: National Academy Press; 1998.
2. Choi SW, Mason JB. Folate and carcinogenesis: an integrated scheme. J Nutr 2000;130:129–32.
3. Chen J, Xu X, Liu AY, Ulrich CM. Folate and cancer: epidemiological perspective. In: Bailey LB, editor. Folate in health and disease. New York: CRC Press; 2009. p. 205–34.
4. Davis SR, Quinlivan EP, Shelnutt KP, Maneval DR, Ghandour H, Capdevila A, et al. The methylenetetrahydrofolate reductase 677C-> T polymorphism and dietary folate restriction affect plasma one-carbon metabolites and red blood cell folate concentrations and distribution in women. J Nutr 2005;135:1040–4.
5. Hubner RA, Muir KR, Liu JF, Sellick GS, Logan RF, Grainge M, et al. Folate metabolism polymorphisms influence risk of colorectal adenoma recurrence. Cancer Epidemiol Biomarkers Prev 2006;15:1607–13.
6. Giovannucci E, Stampfer M, Colditz GA. Multivitamin use, folate and colon cancer in women in the Nurses' Health Study. Ann Intern Med 1998;129:517–24.
7. Ulrich CM, Xu X, Liu A, Chen J. Folate. In: Milner JA, Romagnolo D, editors. Bioactive compounds and cancer. New York: Humana Press; 2010. p. 384–410.
8. Cole BF, Baron JA, Sandler RS, Haile RW, Ahnen DJ, Bresalier RS, et al. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. JAMA 2007;297:2351–9.
9. Stolzenberg-Solomon RZ, Chang SC, Leitzmann MF, Johnson KA, Johnson C, Buys SS, et al. Folate intake, alcohol use, and postmenopausal breast cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Am J Clin Nutr 2006;83:895–4.
10. Ulrich CM. Folate and cancer prevention–Where to next? Counterpoint. Cancer Epidemiol Biomarkers Prev 2008;17:2226–30.
11. Kim Y-I. Folic acid supplementation and cancer risk: point. Cancer Epidemiol Biomarkers Prev 2008;17:2226–30.

Published OnlineFirst July 13, 2011; DOI:10.1158/1055-9965.EPI-10-1352

12. Luebeck EG, Moolgavkar SH, Liu AY, Boynton A, Ulrich CM. Does folic acid supplementation prevent or promote colorectal cancer? Results from model-based predictions. Cancer Epidemiol Biomarkers Prev 2008;17:1360–8.
13. Reed MC, Nijhout HF, Neuhouser ML, Gregory JF 3rd, Shane B, James SJ, et al. A mathematical model gives insights into nutritional and genetic aspects of folate-mediated one-carbon metabolism. J Nutr 2006;136:2653–61.
14. Nijhout HF, Reed MC, Budu P, Ulrich CM. A mathematical model of the folate cycle–new insights into folate homeostasis. J Biol Chem 2004;279:55008–16.
15. Nijhout HF, Reed MC, Anderson DF, Mattingly JC, James SJ, Ulrich CM. Long-range allosteric interactions between the folate and methionine cycles stabilize DNA methylation reaction rate. Epigenetics 2006;1:81–7.
16. Kim Y-I, Fawaz K, Knox T, Lee YM, Norton R, Arora S, et al. Colonic mucosal concentrations of folate correlate well with blood measurements of folate status in persons with colorectal polyps. Am J Clin Nutr 1998;68:866–72.
17. Kim YI, Fawaz K, Knox T, Lee YM, Norton R, Libby E, et al. Colonic mucosal concentrations of folate are accurately predicted by blood measurements of folate status among individuals ingesting physiologic quantities of folate. Cancer Epidemiol Biomarkers Prev 2001;10:715–9.
18. Kim YI, Baik HW, Fawaz K, Knox T, Lee YM, Norton R, et al. Effects of folate supplementation on two provisional molecular markers of colon cancer: a prospective, randomized trial. Am J Gastroenterol 2001;96:184–95.
19. Moxon D, Raza M, Kenny R, Ewing R, Arozullah A, Mason JB, et al. Relationship of aging and tobacco use with the developement of aberrant crypt foci in a predominantly African-American population. Clin Gastroenterol Hepatol 2005;3:271–8.
20. Meenan J, O'Hallinan E, Scott J, Weir DG. Epithelial cell folate depletion occurs in neoplastic but not mucosa. Gastroenterology 1997;112:1163–8.
21. Powers HJ, Hill MH, Welfare M, Spiers A, Bal W, Russell J, et al. Responses of biomarkers of folate and riboflavin status to folate and riboflavin supplementation in healthy and colorectal polyp patients (The FAB2 Study). Cancer Epidemiol Biomarkers Prev 2007;16:2128–35.
22. Ulrich CM, Neuhouser M, Liu AY, Boynton A, Gregory JF 3rd, Shane B, et al. Mathematical modeling of folate metabolism: predicted effects of genetic polymorphisms on mechanisms and biomarkers relevant to carcinogenesis. Cancer Epidemiol Biomarkers Prev 2008;17:1822–31.
23. Bailey RL, Dodd KW, Gahche JJ, Dwyer JT, McDowell MA, Yetley EA, et al. Total folate and folic acid intakes from foods and dietary supplements in the United States: 2003–2006. Am J Clin Nutr 2010;91:231–7.
24. Quinlivan EP, Gregory JF. Reassessing folic acid consumption patterns in the United States (1999–2004): potential effect on neural tube defects and overexposure to folate. Am J Clin Nutr 2007;86:1773–9.
25. Yeung LF, Cogswell ME, Carriquiry AL, Bailey LB, Pfeiffer CM, Berry RJ. Contributions of enriched cereal-grain products, ready-to-eat cereals, and supplements to folic acid and vitamin B-12 usual intake and folate and vitamin B-12 status in US children: National Health and Nutrition Examination Survey, 2003–2006. Am J Clin Nutr 2011;93:172–85.
26. Popat S, Chen Z, Zhao D, Pan H, Hearle N, Chandler I, et al. A prospective, blinded analysis of thymidylate synthase and p53 expression as prognostic markers in the adjuvant treatment of colorectal cancer. Ann Oncol 2006;17:1810–7.
27. Popat S, Matakidou A, Houlston RS. Thymidylate synthase expression and prognosis in colorectal cancer: a systematic review and meta-analysis. J Clin Oncol 2004;22:529–36.
28. Ulrich CM, Curtin K, Potter JD, Bigler J, Caan B, Slattery ML. Polymorphisms in the reduced folate carrier, thymidylate synthase, or methionine synthase and risk of colon cancer. Cancer Epidemiol Biomarkers Prev 2005;14:2509–16.
29. Friso S, Choi S-W, Girelli D, Mason JB, Dolnikowski GG, Bagley PJ, et al. A common mutation in the 5,10-methylenetetra-hydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. Proc Natl Acad Sci U S A 2002;99:5606–11.
30. Wagner C. Biochemical role of folate in cellular metabolism. In: Bailey LB, editor. Folate in health and disease. New York: Marcel Dekker; 1995.
31. Bailey RL, Mills JL, Yetley EA, Gahche JJ, Pfeiffer CM, Dwyer JT, et al. Unmetabolized serum folic acid and its relation to folic acid intake from diet and supplements in a nationally representative sample of adults $\leq$ 60 y in the United States. Am J Clin Nutr 2010;92:383–9.