Supplemental Material can be found at:
http://jn.nutrition.org/cgi/content/full/136/10/2653/DC1

The Journal of Nutrition
Methodology and Mathematical Modeling

A Mathematical Model Gives Insights into Nutritional and Genetic Aspects of Folate-Mediated One-Carbon Metabolism[1,2]

Michael C. Reed,[3] H. Frederik Nijhout,[4] Marian L. Neuhouser,[5] Jesse F. Gregory III,[6] Barry Shane,[7] S. Jill James,[8] Alanna Boynton,[5,9] and Cornelia M. Ulrich[5,9]*

[3]Department of Mathematics and [4]Department of Biology, Duke University, Durham, NC 27708; [5]Cancer Prevention Program, Fred Hutchinson Cancer Research Center, Seattle, WA 98109; [6]Food Science and Human Nutrition Department, University of Florida, Gainesville, FL 32611; [7]Department of Nutritional Sciences and Toxicology, University of California, Berkeley, CA 94720; [8]Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR 72202; and [9]University of Washington, Department of Epidemiology and Interdisciplinary Graduate Program in Nutritional Sciences, Seattle WA 98195

Abstract

Impaired folate-mediated 1-carbon metabolism has been linked to multiple disease outcomes. A better understanding of the nutritional and genetic influences on this complex biochemical pathway is needed to comprehend their impact on human health. To this end, we created a mathematical model of folate-mediated 1-carbon metabolism. The model uses published data on folate enzyme kinetics and regulatory mechanisms to simulate the impact of genetic and nutritional variation on critical aspects of the pathway. We found that the model predictions match experimental data, while providing novel insights into pathway kinetics. Our primary observations were as follows: *1*) the inverse association between folate and homocysteine is strongest at very low folate concentrations, but there is no association at high folate concentrations; *2*) the DNA methylation reaction rate is relatively insensitive to changes in folate pool size; and *3*) as folate concentrations become very high, enzyme velocities decrease. With regard to polymorphisms in 5,10-methylenetetrahydrofolate reductase (MTHFR), the modeling predicts that decrease MTHFR activity reduces concentrations of *S*-adenosylmethionine and 5-methyltetrahydrofolate, as well as DNA methylation, while modestly increasing *S*-adenosylhomocysteine and homocysteine concentrations and thymidine or purine synthesis. Decreased folate together with a simulated vitamin B-12 deficiency results in decreases in DNA methylation and purine and thymidine synthesis. Decreased MTHFR activity superimposed on the B-12 deficiency appears to reverse the declines in purine and thymidine synthesis. These mathematical simulations of folate-mediated 1-carbon metabolism provide a cost-efficient approach to in silico experimentation that can complement and help guide laboratory studies. J. Nutr. 136: 2653–2661, 2006.

JN THE JOURNAL OF NUTRITION

Downloaded from jn.nutrition.org by on May 6, 2008

Introduction

Folate plays a critical role in 1-carbon metabolism, biochemical reactions that are related to amino-acid metabolism, nucleotide synthesis, and numerous methyltransferase reactions, including DNA methylation. Folate and nutrients involved in folate-mediated 1-carbon metabolism (FOCM)[10] are involved in the etiology of neural-tube defects (1), colorectal and other types of cancer (2–6), and cardiovascular disease (7–9). Further, chemotherapeutic agents targeting FOCM play a central role in cancer treatment (10,11). FOCM is highly complex; genetic factors (i.e., polymorphisms in folate-dependent enzymes) and dietary influences interact in intricate ways that ultimately influence folate status and disease risk.

Considerable research over the past 20 y has identified important details about FOCM and its regulation. However, a limiting factor of these critical studies is that they have primarily focused on single pathways and/or single reactions, and thus provide no means for understanding the overall functioning of the system. FOCM comprises a complex nonlinear system, which is difficult to capture using purely experimental methods.

[1] This research was supported by NIH grant R01 CA 105437 (C.M.U.) and NSF grant DMS 0109872 (M.C.R.)

[2] More detailed information on the model parameters and characteristics is available with the online posting of this paper at jn.nutrition.org.

[10] Abbreviations used: 5,10-CH_2-THF, 5,10-methylenetrahydrofolate; 5mTHF, 5-methyltetrahydrofolate; AICART, aminoimidazolecarboxamide ribonucleotide transferase; BHMT, betaine-homocysteine methyltransferase; CBS, cystathionine β-synthase; DHFR, dihydrofolate reductase; DNMT, DNA-methyltransferase; FOCM, folate-mediated 1-carbon metabolism; FTD, 10-formyltetrahydrofolate dehydrogenase; FTS, 10-formyltetrahydrofolate synthase; GNMT, glycine *N*-methyltransferase; Hcy, homocysteine; K_i, inhibition constant; K_m, Michaelis-Menten constant; MAT, methionine adenosyl transferase; METin, rate of methionine input; MS, methionine synthase; MTD, 5,10-methylenetetrahydrofolate dehydrogenase; MTCH, 5,10-methylenetetrahydrofolate cyclohydrolase; MTHFR, 5,10-methylenetetrahydrofolate reductase; PLP, pyridoxal phosphate; SAH, S-adenosylhomocysteine; SAHH, S-adenosylhomocysteine hydrolase; SAM, S-adenosylmethionine; THF, tetrahydrofolate; TS, thymidylate synthase; V_{max}, maximum velocity of reaction.

* To whom correspondence should be addressed. E-mail: nulrich@fhcrc.org.

0022-3166/06 $8.00 © 2006 American Society for Nutrition.

Manuscript received 24 April 2006. Initial review completed 1 June 2006. Revision accepted 15 July 2006.

Mathematical modeling is an approach that has been particularly useful in the study of complex nonlinear biological systems (12,13) and which has been used for individual components of FOCM (14–16).

Building on initial modeling of specific cycles of this pathway (14,15), we developed a mathematical model of the complete cytosolic FOCM, which uses information on folate-enzyme kinetics and regulatory mechanisms to predict the impact of genetic and nutritional variation. We used the model presented here to illustrate how our predictions match experimentally obtained data and provide information on metabolic processes. Throughout the study, we used exactly the same model, and tested the effects of altering particular parameter values or inputs that correspond to these specific biological situations or experiments. Our predictions were generally consistent with those from the experimental literature, which suggests that this simulation model is a valid tool for in silico investigations of FOCM.

Methods

Our objective was to develop a mathematical model that simulates the multiple, interconnected biochemical reactions in folate-mediated 1 carbon metabolism (**Fig. 1**). Our general approach to the model building used integrated information from 3 sources of published mammalian data: *1*) intracellular concentrations of folate-related substrates [e.g., tetrahydrofolate (THF), *5*-methyltetrahydrofolate (5mTHF), *S*-adenosylmethionine (SAM)]; *2*) kinetics of enzymes in the folate and methionine cycles; and *3*) folate enzyme regulatory mechanisms. The values of the kinetic constants reported in the literature were obtained from a variety of species and tissues, including cell lines, and were obtained under a variety of experimental conditions. Thus, it is not surprising that the reported ranges for individual parameters are quite large; we chose values within the published ranges (**Table 1**).

We have already published mathematical models of the folate cycle (15) and the methionine cycle (14,17). For this study, we combined our models for the folate and methionine cycles to build a combined model for intracellular hepatic FOCM. We investigated how the mutual influences of the folate and methionine cycles affect the overall functioning of FOCM (Fig. 1). The 2 cycles are connected, not only by the methionine synthase (MS) reaction, but also by 2 "long-range" interactions: 5,10-methylenetetrahydrofolate reductase (MTHFR) is inhibited by SAM, and glycine *N*-methyltransferase (GNMT) is inhibited by 5mTHF. The other long-range interactions in the model are the inhibition of betaine-homocysteine methyltransferase (BHMT) by SAM and the activation of cystathionine β-synthase (CBS) by SAM. The kinetics of these long-range interactions were taken from the literature as described in (17). There is contradictory evidence about the inhibition of BHMT by SAM, as there are positive (18) and negative (19) reports. We followed the evidence of (18), but found that, because the inhibition is only effective at very high SAM concentrations, the presence or absence of the inhibition had little effect on our results, except for exceptionally high methionine loads.

Because the combined model contained new interactions among enzymes and substrates in the folate and methionine cycles that were absent from the earlier models, some of the substrates developed aberrant concentrations. We therefore modified somewhat, within the published ranges, the V_{max} and K_i values for MTHFR, CBS, MS, GNMT, and BHMT, so that the resulting concentrations of substrates at equilibrium corresponded to those obtained experimentally. We also adjusted the inhibition of DNA-methyltransferase (DNMT) and GNMT by *S*-adenosylhomocysteine (SAH), i.e., lowered the DNMT- and GNMT-specific K_i values (20), and increased the V_{max} of the backward reaction of serine hydroxymethyltransferase (SHMT; glycine to serine). The V_{max} values of thymidylate synthase (TS) and dihydrofolate reductase (DHFR) were increased 100-fold to mimic the conditions observed during cell division (15,21,22). More information about the details of the model is available as Online Supporting Material.

Some substrates and products of FOCM are able to regulate distant enzymes in the network; these mechanisms were modeled as in Nijhout et al. (17). The regulation of MTHFR by SAM and SAH concentrations was obtained by multiplying the V_{max} of MTHFR by $10/(10 + [SAM] - [SAH])$, corresponding to the experiments by Jencks and Matthews (23) and Yamada et al. (24). We scaled these allosteric interactions so that they equal 1 when the methionine input is 100 μmol/(L · h).

The model calculations assume that the normal mean methionine input to the folate pool is 100 μmol/(L · h), based on the work of Storch et al. (25). Their work demonstrated that, during fasting, the net input to the methionine pool is ~4 μmol/(kg · h) (from protein breakdown), which is approximately equivalent to an input of 50 μmol/(L · h) to the liver. Conversely, during and immediately after feeding, the methionine input to the liver is ~4 times as high, or ~200 μmol/(L · h). Assuming that the fasting state lasts for 16 h/d and the postprandial state lasts for 8 h/d in humans, then over a 24-h period the mean methionine input to the liver is ~100 μmol/(L · h).

Our prediction of the DNA methylation reaction rate (= methylation rate) was based on the kinetic characteristics published for the

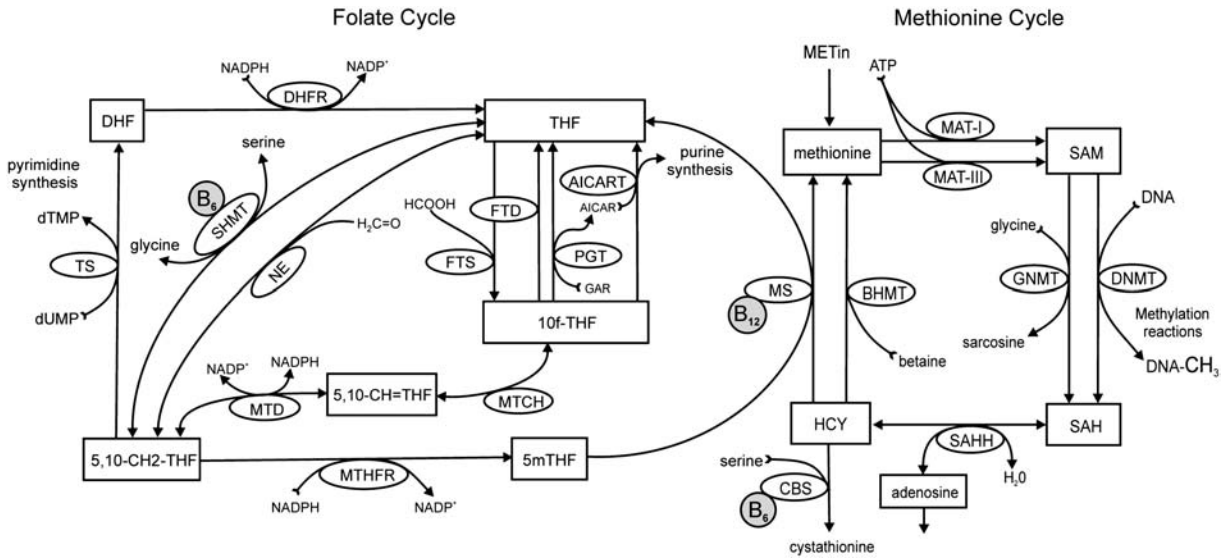

Figure 1 The reaction scheme for FOCM modeled in this article. The substrates are enclosed in rectangular boxes and the enzymes in ellipses; vitamin cofactors are enclosed in shaded circles.

Downloaded from jn.nutrition.org by on May 6, 2008

TABLE 1 Kinetic parameters used in the mathematical model of FOCM[1]

Enzyme	Parameter	Value	Units
CBS	V_{max}	90,000	μmol/(L · h)
BHMT	V_{max}	375	μmol/(L · h)
MS	V_{max}	525	μmol/(L · h)
MTHFR	V_{max}	5000	μmol/(L · h)
DNMT	$K_{i,SAH}$	0.84	μmol/L
GNMT	$K_{i,SAH}$	0.84	μmol/L
	V_{max}	288	μmol/(L · h)
TS	V_{max}	5000	μmol/(L · h)
DHFR	V_{max}	5000	μmol/(L · h)
SHMT	*"Gly → Ser"*		
	V_{max}	320,000	μmol/(L · h)
	$K_{m,5;10\text{-}CH2\text{-}THF}$	3000	μmol/L
CH2	$[H_2C = O]$	500	μmol/L
FTS	[HCOOH]	500	μmol/L

[1] The other kinetic parameters are as described in Nijhout et al. (15,17). For complete details, see the online supporting material.

maintenance DNA methyltransferase (DNMT1) (20). Multiple other methyltransferase reactions run in parallel to the DNMT1 reaction. The majority of methyltransferases have low K_m values for SAM and low K_i values for SAH and, therefore, the reactions they catalyze will behave similarly to DNMT1. DNA methylation depends on the availability of methyl groups, the regulation of the DNMT reaction, and the accessibility of cytosine substrates that is controlled by histones and other DNA-binding proteins. Here we were solely concerned with the first 2 mechanisms and assumed that the accessibility of methylation sites was constant. In the context of our kinetic model, methylation rate represents the reaction flux, whereas most DNA methylation assays refer to relative genome-wide DNA methylation level or the number of cytosine methyl groups at specific locations.

In the folate pool experiments, we changed the total folate pool and computed how the concentrations and reaction rates change. An input parameter was the total folate concentration of the cell; the model then determined the steady-state values of each of the individual folate metabolites. The model showed that, after alteration of the folate pool, the folate metabolites reached their new equilibrium values within an hour. To test the effects of polymorphisms and vitamin deficiencies, we altered the V_{max} values of the corresponding enzymes. To simulate methionine loading we varied the methionine input as a function of time and computed the time courses of the concentrations and reaction rates. All calculations were completed using MatLab (version 7.0, The Mathworks).

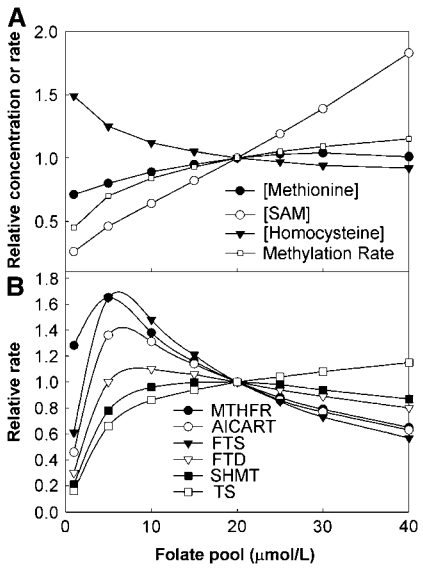

Figure 2 Effect of total folate pool on concentrations and reaction velocities. Metabolites in the methionine cycle and the rate of the DNA methylation reaction (DNMT in Fig. 1), as functions of the total folate pool, are shown in *A*. The concentration of SAM is a linear function of total folate, but the DNA methylation rate was quite stable as total folate varied. The reaction rates in the folate cycle, as functions of the total folate pool, are shown in *B*. Because of the the inhibitory binding of folates to folate enzymes, the reaction velocities were relatively stable as total folate varied between 10 and 30 μmol/L.

Results and Discussion

Consequences of variation in the total folate pool. We computed how the substrate concentrations and reaction velocities at equilibrium change when the total amount of folate is changed (**Fig. 2**). We used 20 μmol/L as a standard cellular folate concentration in the simulations (15,26). As expected, the Hcy concentration increased as total folate fell below this normal concentration, but at higher folate concentrations, Hcy was not influenced by the folate pool. Intracellular Met concentrations are relatively insensitive to variation in total folate, but the concentration of SAM had a strong linear association with total folate concentration (Fig. 2*A*). Because SAM is the principal substrate for cellular methylation reactions, one might expect that the overall methylation rate would be a function of SAM concentration and, therefore, the total folate pool. However, the simulations suggested instead that allosteric interactions between the folate and methionine cycles stabilize the DNMT reaction rate against variations in methionine input and folate pool size (17). Here, as previously (17), we assumed that the availability of CpG methylation sites was constant, and the DNMT reaction rate thus represents the rate of the DNMT reaction when it is unconstrained by the accessibility of methylation sites on the DNA. There were only modest changes in the DNMT reaction rate (Fig. 2*A*), with an increase in folate concentration from 10 μmol/L to 40 μmol/L, whereas SAM increased linearly ~3-fold. Note that as the total folate pool decreased from 20 μmol/L to 1 μmol/L, the methylation rate declined only by 50%. The DNMT reaction rate remained stable at both high folate and SAM concentrations because most methyl-transferase reactions have low K_m values and therefore saturate as SAM increases.

Understanding and modeling these interrelated reactions is complicated because many of the substrates in the folate cycle are also tight-binding inhibitors of enzymes in the folate cycle (26–28). In the absence of such binding, reaction velocities and substrate concentrations would vary in direct proportion to folate pool size (15). Thus, the inhibitory binding causes a remarkable homeostasis in the enzyme velocities of the folate cycle; as total folate decreases, the dissociation of folate-enzyme complexes increases both the amount of active enzyme and additional free folate (15). This mechanism has an important consequence: namely, as folate concentrations become very high, enzyme inhibition increases, and reaction velocities decrease.

Figure 2*B* illustrates the simulated reaction velocities of enzymes in the folate cycle as a function of total folate concentration. Note that there is a fairly wide range of folate concentrations centered at 20 μmol/L, in which the velocities are relatively stable. As the folate pool decreased, some enzyme velocities remained relatively unchanged [SHMT, TS, and 10-formyltetrahydrofolate dehydrogenase (FTD)], whereas MTHFR, aminoimidazolecarboxamide ribonucleotide transferase (AICART), and 10-formyltetrahydrofolate synthase (FTS) showed a sharp increase in velocity. As folate concentrations fell

JN THE JOURNAL OF NUTRITION

Downloaded from jn.nutrition.org by on May 6, 2008

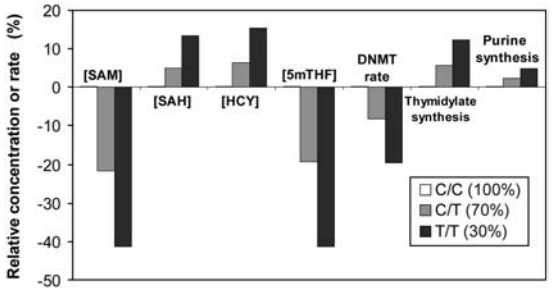

Figure 3 Effect of *MTHFR* C677T polymorphism on modeled hepatic concentrations and reaction rates. The Y axis shows the % change relative to the *MTHFR* C/C genotype (wildtype), assuming 70% enzyme activity for C/T (gray bars), and 30% enzyme activity for T/T (black bars). For a comparison of these model results to epidemiological and experimental results, see the discussion in the text.

below 5 μmol/L, all enzyme velocities declined precipitously. The folate concentrations associated with both peak velocity and the rate of decline depend on the K_i of the inhibition and on the K_m of the substrate. Although substrate concentrations are well known, there is little information on the K_i values; in these simulations we set all K_i values at 1. All of the velocity curves started at 0 when total folate was 0, rose as folate rose, and then continued to decline as folate concentrations became larger. The shapes of the velocity curves were different because the K_m values of the substrates differed. For more details see our discussion in (17).

MTHFR *C677T polymorphism.* The 677TT genotype of the *MTHFR* gene has been associated with an increase in homocysteine (Hcy) (29–38) and an increased risk for neural tube defects (39–47) and cardiovascular disease (7,40,48–51), but possibly a reduced risk for colorectal and hematopoietic malignancies (2,6,52,53), with strong evidence for gene-environment interactions (4,54). The variant allele decreases the activity of MTHFR by ~30% in heterozygotes (CT) and 70% in homozygote variants (TT) (55), and these effects may depend on folate status (56). **Figure 3** illustrates the predicted relative changes at 20 μmol/L folate in the concentrations of SAM, SAH, and Hcy and in the rates of DNA methylation, thymidine synthesis, and purine synthesis as a consequence of these genotypic changes in enzyme function. The simulations suggest that the variant allele decreases the concentrations of SAM and 5mTHF and the DNA methylation rate, and modestly increases the concentrations of SAH, Hcy, and the rates of thymidine and purine synthesis.

The model-predicted changes are comparable to those observed for *MTHFR* genotypes in human populations. For example, homocysteine concentrations have been studied in a variety of populations, including Americans (31,32), Singapore Chinese (33), Europeans (29,34–37), and Japanese (38). These studies have consistently shown that Hcy levels are higher in individuals with the variant (TT) genotype compared with those with the wild type (CC) genotype. However, the magnitude of difference varies across studies, and probably reflects variation in folate (or B-vitamin) status and genetic factors. Most studies report that those with the TT genotype have Hcy concentrations that are 25–35% higher than CC individuals (31–33,36). The association between the TT genotype and Hcy levels is even more pronounced under conditions of folate deficiency, because the thermolability of the MTHFR is increased. We simulated this decrease in MTHFR activity using the data of Guenther et al. (56), and confirmed with the model that the effect of the TT genotype was indeed larger under conditions of folate deficiency: cellular free Hcy concentrations increased by 29% among those with TT genotypes (in relation to CC) when modeled at 10 μmol/L folate, compared with an increase of 15% at 20 μmol/L folate. Note that the model predictions refer to intracellular free homocysteine.

Other biomarkers of folate metabolism were also examined in relation to the *MTHFR* genotypes. For example, studies have found an increase in purine synthesis (57) and a decrease in genomic DNA methylation (58–60) among those with the variant genotype, compared with wild type, which is consistent with the results predicted by our model.

The model predictions, with respect to *MTHFR*, are generally supported by recent empirical data. For example, Quinlivan and colleagues reported that reduction in the MTHFR reaction rate reduced the concentration of the product 5mTHF and increased the concentrations of the other folate substrates. Together, these events increased the rates of thymidine and purine synthesis (57). Our model predicted that the reduction in the 5mTHF concentration slows the remethylation of homocysteine to methionine, thereby increasing the concentration of Hcy and SAH, and decreasing the concentration of SAM. This is inconsistent with observations of Davis et al. who found that homocysteine was elevated but total remethylation was unchanged in a study of human folate depletion (61). However, effects on transsulfuration were not measured. Certainly, further work needs to be done to establish the exact relation between MTHFR, remethylation, and homocysteine production as a product of transmethylation. More extensive simulations of the effects of polymorphisms in FOCM under varying folate status will be the subject of a future investigation.

Vitamin B-12 deficiency. Vitamin B-12 is a critical cofactor of methionine synthase. Vitamin B-12 is obtained from animal products in the diet and its absorption is dependent upon proper gastric-acid secretion, as well as an intact and functioning small intestine, because the site of absorption is very specific. Vegans and the elderly are at particular risk of vitamin B-12 deficiency (62). The primary clinical manifestations of vitamin B-12 deficiency are megaloblastic anemia, neurological deficits, vascular disease and gastrointestinal disturbances (63–65). Details about the synergistic relation between folate and vitamin B-12 and the health consequences of vitamin B-12 deficiency, particularly in the elderly, have been published (8,66,67).

We modeled the interaction of vitamin B-12 with folate status by simulating a reduction of the V_{max} of MS to 10% of its normal value, which is shown in scenarios 1 and 2 of **Table 2**. The reduction to 10% is consistent with observations in patients with severe vitamin B-12 deficiency (68) and it reduced the overall velocity of the MS reaction to 25–30% of its normal value. As expected, the concentration of 5mTHF increased nearly 4-fold, which would cause an effective methyl trap in vivo. The reduction in the other folate substrates was associated with 50% and 73% decreases in purine and thymidine synthesis, respectively. The model predicted that the concentrations of methionine and SAM decreased, albeit modestly, likely because fewer methyl groups were being recirculated. The DNA methylation rate, however, was not changed substantially by variation in MS activity, perhaps because the methylation is stabilized by long-range interactions between the folate and methionine cycles (17).

Long-term B-12 deficiency reduces tissue levels of folate (69). Scenario 3 in Table 2 shows a simulation of a typical B-12 deficiency in conjunction with a low-folate status, which was induced as a result of the methyl trap. As expected, a low-folate

Downloaded from jn.nutrition.org by on May 6, 2008

TABLE 2 Interactions between vitamin B-12 and folate status

	Scenario[1]				
	1	2	3	4	5
Description	Normal	Decreased MS activity	Decreased folate input, decreased MS activity	Decreased MS and MTHFR activity	Increased folate input, decreased MS activity
Model inputs					
Folate, $\mu mol/L$	20	20	10	20	40
MS activity	Normal	10%	10%	10%	10%
MTHFR activity	Normal	Normal	Normal	10%	Normal
Model outputs					
Methionine	48.0	31.6	30.7	30.4	32.3
SAM	64.4	62.0	46.1	41.4	86.3
Hcy	1.11	1.12	1.21	1.25	1.06
5,10-CH_2-THF	0.90	0.24	0.11	0.78	0.49
5mTHF	4.02	15.3	7.8	6.06	30.7
THF	8.01	2.20	0.99	6.87	4.48
5mTHF: THF	0.50	6.95	7.88	0.88	6.85
DNA methylation	132.4	132.0	118.1	112.5	144.3
Purine synthesis	463.9	229.6	128.9	436.3	350.5
Thymidylate synthesis	230.2	62.9	28.5	200.6	127.9

[1] Scenario 1 shows the values of selected concentrations and reaction rates when total folate, MS, and MTHFR were normal. In scenario 2, vitamin B-12 deficiency is simulated by lowering the V_{max} of the MS reaction to 10% of its normal value. Scenario 3 describes the effect of vitamin B-12 deficiency in the presence of low folate. Scenarios 4 and 5 show that the resulting methyl trap could be partly alleviated if one added a MTHFR deficiency or increased total folate.

status exacerbated the vitamin B-12 induced decrease in thymidine and purine synthesis.

Scenario 4 in Table 2 simulated the influence of a reduction of MTHFR activity to 10% of normal in the presence of the 10% MS deficiency. This is a notable simulation because the effect of a loss of MS activity, whether it is caused by a mutation or a dietary deficiency of vitamin B-12, can be partly alleviated by a mutation that reduces the activity of MTHFR (B. Shane, unpublished data). The simulation result shows that *1*) the methyl trap was alleviated; *2*) there was a nearly normal balance among the folate substrates; and *3*) thymidine and purine synthesis returned to concentrations comparable to the normal conditions. Note that under this scenario of decreased MS activity, Hcy increased ~12%, whereas the concentrations of methionine, SAM and DNA methylation decreased by 63%, 64%, and 15%, respectively.

Elevated circulating concentrations of folate can mask a vitamin B-12 deficiency (70). We simulated a scenario mimicking high intracellular folate concentrations, a situation that may arise from use of high-dose dietary supplements containing folate. We raised the total folate level from the normal 20 μmol/L to 40 μmol/L (Table 2, scenario 2 compared with scenario 5), which alleviated some of the adverse consequences of induction of vitamin B-12 deficiency. Thus, for cellular processes that depend on these reactions and substrates, the effect of the vitamin B-12 deficiency can indeed be partially masked by high intakes of folate. Further, comparing scenarios 2 and 5, only the methionine concentration and the ratio of 5mTHF to THF were not changed by folate supplementation in the presence of vitamin B-12 deficiency.

The effect of betaine on homocysteine. Several studies have examined the relation among betaine concentration, homocysteine concentration, and folate status (71–73). Betaine, a choline degradation product, is a normal dietary constituent and also arises from phosphotidylcholine turnover (74). Betaine is also used, together with folate, to treat the severe hyperhomocysteinemia of CBS deficiency. In experimental rodents, the BHMT reaction, although restricted to the liver, is quantitatively more important than folate-dependent remethylation in the reconversion of homocysteine to methionine (B. Shane, unpublished data). The extent to which the BHMT reaction, which typically occurs in liver and kidney in humans, is responsible for homocysteine remethylation in humans is not well understood. Recent studies in mouse models with disruptions in methyltransferases involved in phosphatidylcholine synthesis suggest that methyl group utilization by these reactions may have been greatly underestimated (75,76). In fact, they may represent the major methyl group sinks in metabolism and the major producers of whole body homocysteine (75,76). Under steady state conditions, the choline degradation pathway allowed the possibility of recovery of these methyl groups, with one arising from the BHMT reaction and 2 coming from folate-dependent reactions via dimethylglycine and sarcosine dehydrogenases (77). The extent to which the latter can provide substrates for remethylation would be dependent on folate status.

Published studies have reported the following general findings with regard to the relation of betaine to other substrates in FOCM: *1*) homocysteine concentration is inversely related to betaine concentration; *2*) homocysteine concentration is sensitive to betaine status at low folate status but relatively insensitive at normal and high folate status; and *3*) folic acid supplementation increases betaine concentration (71–73). The results of simulations with our model and plots of homocysteine concentration as a function of betaine status (0.5 = low, 1 = normal, 2 = high) for different relative folate concentrations are shown in **Figure 4***A*. As expected, higher folate status was always inversely associated with homocysteine concentrations (Fig. 4*A*, negative slopes). Moreover, the slopes of the curves became progressively more negative as folate status decreased, confirming observation *2*) (Fig. 4*A*). We tested observation *3*) with our model by raising folate status and found that the reaction rate for BHMT reaction decreased and the betaine concentration increased in a dose-dependent manner (simulations not shown). The explanation for

Downloaded from jn.nutrition.org by on May 6, 2008

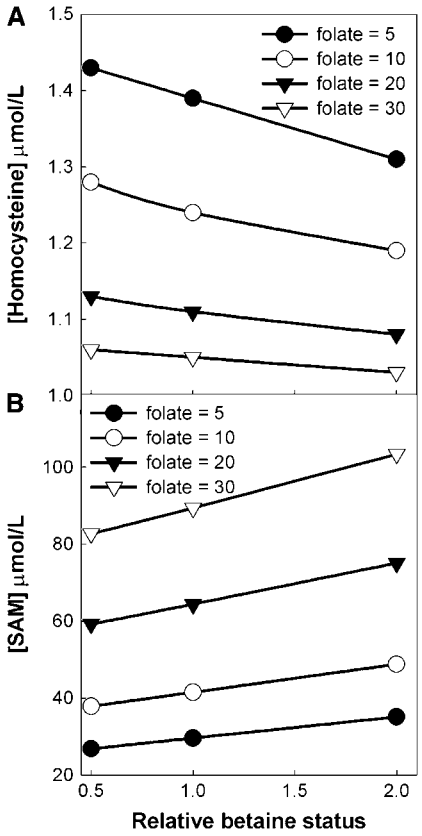

Figure 4 Effect of betaine and folate status on the hepatic concentration of homocysteine and SAM. The amount of betaine affected the velocity of the BHMT reaction. We simulated variation in betaine availability by altering the V_{max} of the BHMT reaction. Betaine status = 1.0 is normal. Relative betaine status indicates the increase or decrease in V_{max} from its normal value.

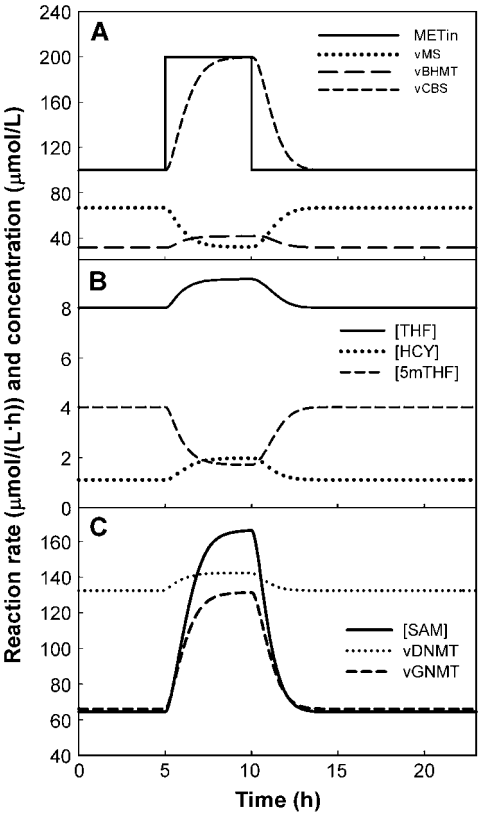

Figure 5 Effect of methionine loading. We simulated a bolus of methionine by doubling the methionine input (METin) for 5 h (*A*). As methionine input went up, the increase in SAM activated CBS and inhibited BHMT (*A*), thus increasing the removal of excess methyl groups from the methionine cycle. The dramatic increase in SAM (*C*) caused inhibition of MTHFR, resulting in a decline of 5mTHF (*B*). Because the MS reaction slowed (*A*), the concentration of homocysteine rose (*B*). The drop in 5mTHF released the inhibition of GNMT, allowing the GNMT reaction to carry most of the excess methyl groups. Thus, the DNA methylation rate (vDNMT) changed only slightly (*C*). Concentrations are in brackets and reaction velocities are indicated by the prefix v.

this effect is that high folate status drove the methionine synthase reaction faster, which increased SAM concentration (Fig. 4*B*). This in turn inhibited BHMT so less betaine was metabolized, thus increasing the betaine concentration.

Methionine loading. Methionine loading causes an increase in hepatic SAM and plasma homocysteine, and a relative stimulation of the transsulfuration pathway. The increase in plasma homocysteine following a methionine load is primarily influenced by vitamin B-6 status, rather than folate or vitamin B-12, as the enzymes of the transsulfuration pathway depend on pyridoxal phosphate (PLP). Thus, the methionine load test has been used to assess vitamin B-6 status. The transsulfuration pathway also is stimulated following a protein containing meal (25,78).

To simulate the effect of methionine loading on folate and methionine metabolism, we doubled the normal rate of methionine input [METin = 100 μmol/(L · h)] to 200 μmol/(L · h) for a 5-h period (**Fig. 5**). Fig. 5*A* shows the effect of the methionine load on the transsulfuration and remethylation reactions. As expected, Hcy rose (Fig. 5*B*). However, V_{MS} declined dramatically, because its other substrate, 5mTHF, declined dramatically (Fig. 5*A*). This is likely due to the steep rise in SAM (Fig. 5*C*) that inhibited the synthesis of 5mTHF by inhibiting MTHFR. There were 2 competing effects on V_{BHMT}: the rise in its substrate, Hcy, and inhibition of the enzyme by SAM. The data suggest that the former has a larger magnitude of effect than the latter (Fig. 5*A*).

Although the rate of the MS reaction decreased during the methionine load, the concentration of its product, THF, rose (Fig. 5*B*). In the model simulations, this was because the inhibition of MTHFR caused the concentration of 5,10-CH_2-THF to rise, which directed folate toward THF through the 5,10-methylenetetrahydrofolate dehydrogenase (MTD)-5,10-methylenetetrahydrofolate cyclohydrolase (MTCH) pathway.

One might expect that the dramatic increase in SAM during methionine loading would result in an equally dramatic increase in the rate of DNA methylation (Fig. 5*C*). However, methylation remained remarkably stable. This may be due to the long-range interactions between the folate and methionine cycles (17) and because the increased flux of methyl groups was almost entirely carried by V_{GNMT} (Fig. 5*C*), a mechanism first proposed by Wagner et al. (79).

General discussion. We found that our mathematical model of FOCM, which is based on established physiology and biochemistry, can reliably reproduce experimental data from both humans and rodents. Model predictions help explain clinical observations about folate and nutrients relevant to folate metabolism, as well as experimental data from both humans and rodents. Initial modeling has given insights on the mechanisms behind folate homeostasis (15) and the stability of DNA methylation in the face of methionine fluctuations (17). The availability of this functioning mathematical model allows us to easily perform in silico experiments to test hypotheses and provide guidance for experimental studies.

Downloaded from jn.nutrition.org by on May 6, 2008

Our model will be particularly useful for providing initial predictions on the combined effects of variation in genetic and nutritional factors on biomarkers of disease risk. Genotype-targeted human feeding studies are expensive and labor intensive, and may be limited by small sample size (57,61,80,81). Whereas these feeding studies provide a gold standard design for understanding mechanisms related to gene-nutrient interactions, the mathematical model will help identify which specific interactions may be most promising for these costly investigations in the human experimental setting. Mathematical models are not intended to replace experimental methods. Rather, these models can be a valuable tool for laboratory researchers and may be used to provide pilot data for human studies.

Our current model is based on data from rats and humans and reflects hepatic FOCM. One of our next steps will be to adapt the model to nonhepatic FOCM, because FOCM in epithelia, such as colonic mucosa, will be important for disease risk estimation. Folate status and folate-metabolizing polymorphisms are clearly linked to colorectal carcinogenesis. We anticipate that the modeling will help us understand some of the gene-gene and gene-environment interactions that have been observed in studies of colorectal cancer or its precursors (4,82). In addition, we anticipate that FOCM modeling outputs will enable us to integrate more biologic information in epidemiologic data analyses, as part of hierarchical modeling structures (83,84).

Further, we plan to extend the model to include mitochondrial compartmentalization. Others have shown that the exchange between mitochondrial and cytosolic folate metabolites and amino acids is an important aspect of intracellular folate metabolism, and that polymorphisms in mitochondrial enzymes should also be considered (26,49,85). We also plan to create a detailed model of folate and amino acid transport between the blood and liver cells (86). This will enable us to infer cellular FOCM status from measurements made in the blood.

We recognize that the biochemical input parameters used in the model often were determined from a wide range of experimental values. Model-derived information can be used to identify components that need to be measured with high accuracy in experimental settings. By conducting a sensitivity analysis using the mathematical model, we are able to determine how sensitive any specific flux, metabolite concentration, or other biomarker is to changes in inputs or enzyme activities. We found, for instance, that some properties of FOCM are extremely insensitive to changes in input parameters (e.g., DNA methylation rate in Figs. 2 and 5), whereas others are sensitive. The model can be used to determine which parameter changes affect biologically relevant processes, such as nucleotide synthesis. This will help to identify components that easily alter the system's functioning as promising targets for epidemiological investigation and therapeutic interventions.

In summary, we developed an initial model of FOCM that can reproduce experimental conditions. We anticipate that in silico predictions will complement, and help direct, experimental or epidemiologic investigations and enhance our understanding of this important biologic pathway.

Literature Cited

1. Mitchell LE. Epidemiology of neural tube defects. Am J Med Genet C Semin Med Genet. 2005;135:88–94.
2. Little J, Sharp L, Duthie S, Narayanan S. Colon cancer and genetic variation in folate metabolism: the clinical bottom line. J Nutr. 2003;133:3758S–66S.
3. Giovannucci E. Epidemiologic studies of folate and colorectal neoplasia: a review. J Nutr. 2002;132:2350S–5S.
4. Ulrich CM. Nutrigenetics in cancer research–folate metabolism and colorectal cancer. J Nutr. 2005;135:2698–702.
5. Rampersaud GC, Bailey LB, Kauwell GP. Relationship of folate to colorectal and cervical cancer: Review and recommendations for practitioners. J Am Diet Assoc. 2002;102:1273–82.
6. Robien K, Ulrich CM. 5,10-methylenetetrahydrofolate reductase polymorphisms and leukemia risk. Am J Epidemiol. 2003;157:571–82.
7. Lewis SJ, Ebrahim S, Davey Smith G. Meta-analysis of MTHFR 677C→T polymorphism and coronary heart disease: does totality of evidence support causal role for homocysteine and preventive potential of folate? BMJ. 2005;331:1053.
8. Stover PJ. Physiology of folate and vitamin B12 in health and disease. Nutr Rev. 2004;62:S3–12.
9. Moat SJ, Lang D, McDowell IF, Clarke ZL, Madhavan AK, Lewis MJ, Goodfellow J. Folate, homocysteine, endothelial function and cardiovascular disease. J Nutr Biochem. 2004;15:64–79.
10. Ulrich CM, Robien K, McLeod HL. Cancer pharmacogenetics: polymorphisms, pathways and beyond. Nat Rev Cancer. 2003;3:912–20.
11. Robien K, Boynton A, Ulrich CM. Pharmacogenetics of folate-related drug targets in cancer treatment. Pharmacogenomics. 2005;6:673–89.
12. Murray JD. Mathematical Biology. Berlin: Springer Verlag; 1989.
13. Edelstein-Keshet L. Mathematical models in biology. New York: Random House; 1988.
14. Reed MC, Nijhout HF, Sparks R, Ulrich CM. A mathematical model of the methionine cycle. J Theor Biol. 2004;226:33–43.
15. 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.
16. Prudova A, Martinov MV, Vitvitsky VM, Ataullakhanov FI, Banerjee R. Analysis of pathological defects in methionine metabolism using a simple mathematical model. Biochim Biophys Acta. 2005;1741:331–8.
17. Nijhout HF, Reed M, Anderson D, Mattingly J, James SJ, Ulrich CM. Long-range allosteric interactions between the folate and methionine cycles stabilize DNA methylation rate. Epigenetics. 2006;1:81–7.
18. Finkelstein JD, Martin JJ. Methionine metabolism in mammals. Distribution of homocysteine between competing pathways. J Biol Chem. 1984;259:9508–13.
19. Bose N, Greenspan P, Momany C. Expression of recombinant human betaine: homocysteine S-methyltransferase for x-ray crystallographic studies and further characterization of interaction with S-adenosylmethionine. Protein Expr Purif. 2002;25:73–80.
20. Clarke S, Banfield K. S-Adenosylmethionine-dependent methyltransferases. In: Carmel R, Jacobsen DW, editors. Homocysteine in Health and Disease. Cambridge: Cambridge University Press; 2001. p. 63–78.
21. Bjarnason GA, Jordan RC, Wood PA, Li Q, Lincoln DW, Sothern RB, Hrushesky WJ, Ben-David Y. Circadian expression of clock genes in human oral mucosa and skin: association with specific cell-cycle phases. Am J Pathol. 2001;158:1793–801.
22. Wade M, Blake MC, Jambou RC, Helin K, Harlow E, Azizkhan JC. An inverted repeat motif stabilizes binding of E2F and enhances transcription of the dihydrofolate reductase gene. J Biol Chem. 1995;270:9783–91.
23. Jencks DA, Mathews RG. Allosteric inhibition of methylenetetrahydrofolate reductase by adenosylmethionine. Effects of adenosylmethionine and NADPH on the equilibrium between active and inactive forms of the enzyme and on the kinetics of approach to equilibrium. J Biol Chem. 1987;262:2485–93.
24. Yamada K, Chen Z, Rozen R, Matthews RG. Effects of common polymorphisms on the properties of recombinant human methylenetetrahydrofolate reductase. [comment] Proc Natl Acad Sci USA. 2001;98:14853–8.
25. Storch KJ, Wagner DA, Burke JF, Young VR. Quantitative study in vivo of methionine cycle in humans using [methyl-2H3]- and [1–13C]methionine. Am J Physiol. 1988;255:E322–31.
26. Cook RJ. Folate metabolism. In: Carmel R, Jacobsen DW, editors. Homocysteine in Health and Disease. Cambridge: Cambridge University Press; 2001. p. 113–34.
27. Min H, Shane B, Stokstad EL. Identification of 10-formyltetrahydrofolate dehydrogenase-hydrolase as a major folate binding protein in liver cytosol. Biochim Biophys Acta. 1988;967:348–53.
28. Wagner C. Symposium on the subcellular compartmentation of folate metabolism. J Nutr. 1996;126:1228S–34S.

Downloaded from jn.nutrition.org by on May 6, 2008

29. Kluijtmans LA, Young IS, Boreham CA, Murray L, McMaster D, McNulty H, Strain JJ, McPartlin J, Scott JM, Whitehead AS. Genetic and nutritional factors contributing to hyperhomocysteinemia in young adults. Blood. 2003;101:2483–8.
30. Blom HJ. Mutated 5,10-methylenetetrahydrofolate reductase and moderate hyperhomocysteinaemia. Eur J Pediatr. 1998;157:S131–4.
31. McNulty H, McKinley MC, Wilson B, McPartlin J, Strain JJ, Weir DG, Scott JM. Impaired functioning of thermolabile methylenetetrahydrofolate reductase is dependent on riboflavin status: implications for riboflavin requirements. Am J Clin Nutr. 2002;76:436–41.
32. Ma J, Stampfer MJ, Hennekens CH, Frosst P, Selhub J, Horsford J, Malinow MR, Willett WC, Rozen R. Methylenetetrahydrofolate reductase polymorphism, plasma folate, homocysteine, and risk of myocardial infarction in US physicians. [see comments] Circulation. 1996;94:2410–6.
33. Trinh BN, Ong C-N, Coetzee GA, Yu MC, Laird PW. Thymidylate synthase: a novel genetic determinant of plasma homocysteine and folate levels. Hum Genet. 2002;111:299–302.
34. Dekou V, Gudnason V, Hawe E, Miller GJ, Stansbie D, Humphries SE. Gene-environment and gene-gene interaction in the determination of plasma homocysteine levels in healthy middle-aged men. Thromb Haemost. 2001;85:67–74.
35. Dedoussis GV, Panagiotakos DB, Chrysohoou C, Pitsavos C, Zampelas A, Choumerianou D, Stefanadis C. Effect of interaction between adherence to a Mediterranean diet and the methylenetetrahydrofolate reductase 677C→T mutation on homocysteine concentrations in healthy adults: the ATTICA Study. Am J Clin Nutr. 2004;80:849–54.
36. de Bree A, Verschuren WM, Bjorke-Monsen AL, van der Put NM, Heil SG, Trijbels FJ, Blom HJ. Effect of the methylenetetrahydrofolate reductase 677C→T mutation on the relations among folate intake and plasma folate and homocysteine concentrations in a general population sample. Am J Clin Nutr. 2003;77:687–93.
37. Chango A, Potier De Courcy G, Boisson F, Guilland JC, Barbe F, Perrin MO, Christides JP, Rabhi K, Pfister M, et al. 5,10-methylenetetrahydrofolate reductase common mutations, folate status and plasma homocysteine in healthy French adults of the Supplementation en Vitamines et Mineraux Antioxydants (SU.VI.MAX) cohort. Br J Nutr. 2000;84:891–6.
38. Moriyama Y, Okamura T, Kajinami K, Iso H, Inazu A, Kawashiri M, Mizuno M, Takeda Y, Sakamoto Y, et al. Effects of serum B vitamins on elevated plasma homocysteine levels associated with the mutation of methylenetetrahydrofolate reductase gene in Japanese. Atherosclerosis. 2002;164:321–8.
39. De Marco P, Calevo MG, Moroni A, Arata L, Merello E, Finnell RH, Zhu H, Andreussi L, Cama A, Capra V. Study of MTHFR and MS polymorphisms as risk factors for NTD in the Italian population. J Hum Genet. 2002;47:319–24.
40. Ueland PM, Hustad S, Schneede J, Refsum H, Vollset SE. Biological and clinical implications of the MTHFR C677T polymorphism. Trends Pharmacol Sci. 2001;22:195–201.
41. De Marco P, Moroni A, Merello E, de Franchis R, Andreussi L, Finnell RH, Barber RC, Cama A, Capra V. Folate pathway gene alterations in patients with neural tube defects. Am J Med Genet. 2000;95:216–23.
42. Christensen B, Arbour L, Tran P, Leclerc D, Sabbaghian N, Platt R, Gilfix BM, Rosenblatt DS, Gravel RA, et al. Genetic polymorphisms in methylenetetrahydrofolate reductase and methionine synthase, folate levels in red blood cells, and risk of neural tube defects. Am J Med Genet. 1999;84:151–7.
43. Shaw GM, Rozen R, Finnell RH, Wasserman CR, Lammer EJ. Maternal vitamin use, genetic variation of infant methylenetetrahydrofolate reductase, and risk for spina bifida. Am J Epidemiol. 1998;148:30–7.
44. Rizzari C, Valsecchi MG, Conter V. MTHFR 677C→T mutation and neural-tube defects. [letter; comment] Lancet. 1997;350:1479–80.
45. Posey DL, Khoury MJ, Mulinare J, Adams MJ, Jr., Ou CY. Is mutated MTHFR a risk factor for neural tube defects? [letter] Lancet. 1996;347:686–7.
46. Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. Am J Epidemiol. 2000;151:862–77.
47. Boyles AL, Hammock P, Speer MC. Candidate gene analysis in human neural tube defects. Am J Med Genet C Semin Med Genet. 2005;135: 9–23.
48. Rozen M. Polymorphisms of folate and cobalamin metabolism. In: Carmel R, Jacobsen DW, editors. Homocysteine in Health and Disease. Cambridge: Cambridge University Press; 2001. p. 259–69.
49. Lim U, Peng K, Shane B, Stover PJ, Litonjua AA, Weiss ST, Gaziano JM, Strawderman RL, Raiszadeh F, et al. Polymorphisms in cytoplasmic serine hydroxymethyltransferase and methylenetetrahydrofolate reductase affect the risk of cardiovascular disease in men. J Nutr. 2005;135:1989–94.
50. Frederiksen J, Juul K, Grande P, Jensen GB, Schroeder TV, Tybjaerg-Hansen A, Nordestgaard BG. Methylenetetrahydrofolate reductase polymorphism (C677T), hyperhomocysteinemia, and risk of ischemic cardiovascular disease and venous thromboembolism: prospective and case-control studies from the Copenhagen City Heart Study. Blood. 2004;104:3046−51.
51. Schwartz SM, Siscovick DS, Malinow MR, Rosendaal FR, Beverly RK, Hess DL, Psaty BM, Longstreth WT, Jr., Koepsell TD, et al. Myocardial infarction in young women in relation to plasma total homocysteine, folate, and a common variant in the methylenetetrahydrofolate reductase gene. Circulation. 1997;96:412–7.
52. Le Marchand L, Wilkens LR, Kolonel LN, Henderson BE. The MTHFR C677T polymorphism and colorectal cancer: the multiethnic cohort study. Cancer Epidemiol Biomarkers Prev. 2005;14:1198–203.
53. Curtin K, Bigler J, Slattery ML, Caan B, Potter JD, Ulrich CM. MTHFR C677T and A1298C polymorphisms: diet, estrogen, and risk of colon cancer. Cancer Epidemiol Biomarkers Prev. 2004;13:285–92.
54. Ulrich CM, Kampman E, Bigler J, Schwartz SM, Chen C, Bostick R, Fosdick L, Beresford SA, Yasui Y, Potter JD. Colorectal adenomas and the C677T MTHFR polymorphism: evidence for gene-environment interaction? Cancer Epidemiol Biomarkers Prev. 1999;8:659–68.
55. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. [letter] Nat Genet. 1995;10:111–3.
56. Guenther BD, Sheppard CA, Tran P, Rozen R, Matthews RG, Ludwig ML. The structure and properties of methylenetetrahydrofolate reductase from Escherichia coli suggest how folate ameliorates human hyperhomocysteinemia. Nat Struct Biol. 1999;6:359–65.
57. Quinlivan EP, Davis SR, Shelnutt KP, Henderson GN, Ghandour H, Shane B, Selhub J, Bailey LB, Stacpoole PW, Gregory, 3rd JF. Methylenetetrahydrofolate reductase 677C→T polymorphism and folate status affect one-carbon incorporation into human DNA deoxynucleosides. J Nutr. 2005;135:389–96.
58. Stern LL, Mason JB, Selhub J, Choi SW. Genomic DNA hypomethylation, a characteristic of most cancers, is present in peripheral leukocytes of individuals who are homozygous for the C677T polymorphism in the methylenetetrahydrofolate reductase gene. Cancer Epidemiol Biomarkers Prev. 2000;9:849–53.
59. Castro R, Rivera I, Ravasco P, Camilo ME, Jakobs C, Blom HJ, de Almeida IT. 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C→T and 1298A→C mutations are associated with DNA hypomethylation. J Med Genet. 2004;41:454–8.
60. Friso S, Choi SW, Girelli D, Mason JB, Dolnikowski GG, Bagley PJ, Olivieri O, Jacques PF, Rosenberg IH, et al. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. Proc Natl Acad Sci USA. 2002;99:5606–11.
61. Davis SR, Quinlivan EP, Shelnutt KP, Ghandour H, Capdevila A, Coats BS, Wagner C, Shane B, Selhub J, et al. Homocysteine synthesis is elevated but total remethylation is unchanged by the methylenetetrahydrofolate reductase 677C→T polymorphism and by dietary folate restriction in young women. J Nutr. 2005;135:1045–50.
62. Wolters M, Strohle A, Hahn A. Cobalamin: a critical vitamin in the elderly. Prev Med. 2004;39:1256–66.
63. Institute of MedicineDietary Reference Intakes: Thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington, DC: National Academy Press.; 1998.
64. Morris MC, Evans DA, Bienias JL, Tangney CC, Hebert LE, Scherr PA, Schneider JA. Dietary folate and vitamin B12 intake and cognitive decline among community-dwelling older persons. Arch Neurol. 2005;62:641–5.
65. Bleie O, Refsum H, Ueland PM, Vollset SE, Guttormsen AB, Nexo E, Schneede J, Nordrehaug JE, Nygard O. Changes in basal and postmethionine load concentrations of total homocysteine and

Downloaded from jn.nutrition.org by on May 6, 2008

cystathionine after B vitamin intervention. Am J Clin Nutr. 2004;80: 641–8.
66. Huerta JM, Gonzalez S, Vigil E, Prada M, San Martin J, Fernandez S, Patterson AM, Lasheras C. Folate and cobalamin synergistically decrease the risk of high plasma homocysteine in a nonsupplemented elderly institutionalized population. Clin Biochem. 2004;37:904–10.
67. Bailey LB. Folate and vitamin B12 recommended intakes and status in the United States. Nutr Rev. 2004;62:S14–20.
68. Taylor RT, Hanna ML, Hutton JJ. 5-methyltetrahydrofolate homocysteine cobalamin methyltransferase in human bone marrow and its relationship to pernicious anemia. Arch Biochem Biophys. 1974;165:787–95.
69. Herbert V. Folic acid. Annu Rev Med. 1965;16:359–70.
70. Savage DG, Lindenbaum J. Folate-cobalamin interactions. In: Bailey LB, editor. Folate in Health and Disease. New York: Marcel Dekker; 1995. p. 237–85.
71. Holm PI, Bleie O, Ueland PM, Lien EA, Refsum H, Nordrehaug JE, Nygard O. Betaine as a determinant of postmethionine load total plasma homocysteine before and after B-vitamin supplementation. Arterioscler Thromb Vasc Biol. 2004;24:301–7.
72. Holm PI, Ueland PM, Vollset SE, Midttun O, Blom HJ, Keijzer MB, den Heijer M. Betaine and folate status as cooperative determinants of plasma homocysteine in humans. Arterioscler Thromb Vasc Biol. 2005;25:379–85.
73. Melse-Boonstra A, Holm PI, Ueland PM, Olthof M, Clarke R, Verhoef P. Betaine concentration as a determinant of fasting total homocysteine concentrations and the effect of folic acid supplementation on betaine concentrations. Am J Clin Nutr. 2005;81:1378–82.
74. Zeisel SH, Mar MH, Howe JC, Holden JM. Concentrations of choline-containing compounds and betaine in common foods. J Nutr. 2003;133:1302–7.
75. Jacobs RL, Stead LM, Devlin C, Tabas I, Brosnan ME, Brosnan JT, Vance DE. Physiological regulation of phospholipid methylation alters plasma homocysteine in mice. J Biol Chem. 2005;280:28299–305.
76. Stead LM, Brosnan JT, Brosnan ME, Vance DE, Jacobs RL. Is it time to reevaluate methyl balance in humans? Am J Clin Nutr. 2006;83: 5–10.
77. Shane B. Folate, vitamin B12 and vitamin B6. In: Stipanuk MH, editor. Biochemical and Physiological Bases of Human Nutrition. New York: Saunders; 2000. p. 483–518.
78. Storch KJ, Wagner DA, Young VR. Methionine kinetics in adult men: effects of dietary betaine on L-[2H3-methyl-1–13C]methionine. Am J Clin Nutr. 1991;54:386–94.
79. Wagner C, Briggs WT, Cook RJ. Inhibition of glycine N-methyltransferase activity by folate derivatives: implications for regulation of methyl group metabolism. Biochem Biophys Res Commun. 1985;127:746–52.
80. Kauwell GP, Wilsky CE, Cerda JJ, Herrlinger-Garcia K, Hutson AD, Theriaque DW, Boddie A, Rampersaud GC, Bailey LB. Methylenetetrahydrofolate reductase mutation (677C→T) negatively influences plasma homocysteine response to marginal folate intake in elderly women. Metabolism. 2000;49:1440–3.
81. Davis SR, Scheer JB, Quinlivan EP, Coats BS, Stacpoole PW, Gregory, 3rd JF. Dietary vitamin B-6 restriction does not alter rates of homocysteine remethylation or synthesis in healthy young women and men. Am J Clin Nutr. 2005;81:648–55.
82. 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.
83. Ulrich CM, Nijhout HF, Reed MC. Mathematical modeling: epidemiology meets systems biology. Cancer Epidemiol Biomarkers Prev, in press.
84. Ulrich CM, Nijhout HF, Reed MC. Mathematical modeling: epidemiology meets systems biology. Cancer Epidemiol Biomarkers Prev. 2006;15:827–9.
85. Stover PJ, Chen LH, Suh JR, Stover DM, Keyomarsi K, Shane B. Molecular cloning, characterization, and regulation of the human mitochondrial serine hydroxymethyltransferase gene. J Biol Chem. 1997;272:1842–8.
86. Gregory, 3rd JF, Quinlivan EP. In vivo kinetics of folate metabolism. Annu Rev Nutr. 2002;22:199–220.

Downloaded from jn.nutrition.org by on May 6, 2008