A Mathematical Model Gives Insights into Nutritional and Genetic Aspects of Folate-Mediated One-Carbon Metabolism

Michael C. Reed, H. Frederik Nijhout, Marian L. Neuhouser, Jesse F. Gregory III, Barry Shane, S. Jill James, Alanna Boynton and Cornelia M. Ulrich

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) are involved in the etiology of neural-tube defects, colorectal and other types of cancer, and cardiovascular disease. Further, chemo-therapeutic agents targeting FOCM play a central role in cancer treatment. 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-methyleneetrahydrofolate 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.
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 \( V_{\text{max}} \) 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 Ki values (20), and increased the \( V_{\text{max}} \) of the backward reaction of serine hydroxymethyltransferase (SHMT; glycine to serine). The \( V_{\text{max}} \) 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 \( V_{\text{max}} \) of MTHFR by \( 10/10 + [\text{SAM}] - [\text{SAH}] \), 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 (eq methylation rate) was based on the kinetic characteristics published for the

![Folate Cycle](image1)

**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.

2654 Reed et al.
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).

### 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 $\mu$mol/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. 2A). 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. 2A), with an increase in folate concentration from 10 $\mu$mol/L to 40 $\mu$mol/L, whereas SAM increased linearly ~3-fold. Note that as the total folate pool decreased from 20 $\mu$mol/L to 1 $\mu$mol/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 2B 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 $\mu$mol/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

---

**Table 1** Kinetic parameters used in the mathematical model of FOCM

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBS</td>
<td>$V_{max}$</td>
<td>90,000</td>
<td>$\mu$mol/(L·h)</td>
</tr>
<tr>
<td>BHMT</td>
<td>$V_{max}$</td>
<td>375</td>
<td>$\mu$mol/(L·h)</td>
</tr>
<tr>
<td>MS</td>
<td>$V_{max}$</td>
<td>525</td>
<td>$\mu$mol/(L·h)</td>
</tr>
<tr>
<td>MTHFR</td>
<td>$V_{max}$</td>
<td>5000</td>
<td>$\mu$mol/(L·h)</td>
</tr>
<tr>
<td>DNMT</td>
<td>$K_i,SAH$</td>
<td>0.84</td>
<td>$\mu$mol/L</td>
</tr>
<tr>
<td>GNMT</td>
<td>$V_{max}$</td>
<td>288</td>
<td>$\mu$mol/(L·h)</td>
</tr>
<tr>
<td>TS</td>
<td>$V_{max}$</td>
<td>5000</td>
<td>$\mu$mol/(L·h)</td>
</tr>
<tr>
<td>DHFR</td>
<td>$V_{max}$</td>
<td>5000</td>
<td>$\mu$mol/(L·h)</td>
</tr>
<tr>
<td>SHMT</td>
<td>$V_{max}$</td>
<td>320,000</td>
<td>$\mu$mol/(L·h)</td>
</tr>
<tr>
<td>CH2</td>
<td>$K_{HOT,THF}$</td>
<td>3000</td>
<td>$\mu$mol/L</td>
</tr>
<tr>
<td>FTS</td>
<td>$V_{max}$</td>
<td>500</td>
<td>$\mu$mol/(L·h)</td>
</tr>
</tbody>
</table>

1 The other kinetic parameters are as described in Nijhout et al. (15,17). For complete details, see the online supporting material.
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 thrombosis in the elderly, have been published (8,66,67). 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.

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 methyltransferase. 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 $V_{\text{max}}$ 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
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 phosphatidylcholine 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 normal, 1 normal, 2 high) for different relative folate concentrations are shown in Figure 4A. As expected, higher folate status was always inversely associated with homocysteine concentrations (Fig. 4A, negative slopes). Moreover, the slopes of the curves became progressively more negative as folate status decreased, confirming observation 2 (Fig. 4A). 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
this effect is that high folate status drove the methionine synthase reaction faster, which increased SAM concentration (Fig. 4B). 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 \( \text{METin} = 100 \, \text{mmol/(L} \cdot \text{h}) \) to 200 \( \text{mmol/(L} \cdot \text{h}) \) for a 5-h period (Fig. 5A). As methionine input went up, the increase in SAM activated CBS and inhibited BHMT (Fig. 5A), thus increasing the removal of excess methyl groups from the methionine cycle. The dramatic increase in SAM (\( \text{SAM} \)) caused inhibition of MTHFR, resulting in a decline of 5mTHF (\( \text{5mTHF} \)). Because the MS reaction slowed (\( \text{MS} \)), the concentration of homocysteine rose (\( \text{Hcy} \)). 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 \( \text{vDNMT} \) changed only slightly (\( \text{vDNMT} \)). Concentrations are in brackets and reaction velocities are indicated by the prefix v.

**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.

**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 \( V_{\text{max}} \) of the BHMT reaction. Betaine status = 1.0 is normal. Relative betaine status indicates the increase or decrease in \( V_{\text{max}} \) from its normal value.

**Figure 5** Effect of methionine loading. We simulated a bolus of methionine by doubling the methionine input \( \text{METin} \) 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 (\( \text{SAM} \)) 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 \( \text{vDNMT} \) changed only slightly (C). Concentrations are in brackets and reaction velocities are indicated by the prefix v.
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 epidemiologic 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**


