Folate deprivation reduces homocysteine remethylation in a human intestinal epithelial cell culture model: role of serine in one-carbon donation

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Townsend, Justin H., Steven R. Davis, Amy D. Mackey, and Jesse F. Gregory III. Folate deprivation reduces homocysteine remethylation in a human intestinal epithelial cell culture model: role of serine in one-carbon donation. Am J Physiol Gastrointest Liver Physiol 286: G588–G595, 2004. First published November 13, 2003; 10.1152/ajpgi.00454.2003.—Little is known about homocysteine metabolism in intestine. To address this question, we investigated homocysteine metabolism under conditions of folate adequacy and folate deprivation in the Caco-2 cell line, a model of human intestinal mucosal cells. Caco-2 cells were cultured in media enriched with [3-3H]serine and [U-14C]methionine tracers, and enrichments of intracellular free amino acid pools of these amino acids as well as homocysteine, cystathionine, and cysteine were measured by using gas chromatography/mass spectrometry. Homocysteine transsulfuration plus folate-dependent and total remethylation were quantified from these amino acid enrichments. Homocysteine remethylation accounted for 19% of the intracellular free methionine pool in cells cultured with supplemental folate, and nearly all one-carbon units used for remethylation originated from the three carbon of serine via folate-dependent remethylation. Labelling of cystathionine and cysteine indicated the presence of a complete transsulfuration pathway in Caco-2 cells, and this pathway produced 13% of the intracellular free cysteine pool. Appearance of labeled homocysteine and cystathionine in culture medium suggests export of these metabolites from intestinal cells. Remethylation was reduced by one-third in folate-restricted cell cultures (P < 0.001), and only ~50% of the one-carbon units used for remethylation originated from the three carbon of serine under these conditions. In conclusion, the three carbon of serine is the primary source of one-carbon units used for homocysteine remethylation in folate-supplemented Caco-2 cell cultures. Remethylation is reduced as a result of folate restriction in this mucosal cell model, and one-carbon sources other than the three carbon of serine contribute to remethylation under this condition.

methionine; methylation; Caco-2; transsulfuration; serine

FOLATE DEFICIENCY AND GENETIC polymorphisms of several enzymes involved in one-carbon metabolism are linked to cardiovascular disease and certain cancers and particularly colorectal cancer (5, 6, 16, 18). These associations are likely due to the effects of altered folate metabolism on homocysteine remethylation, DNA synthesis, and DNA methylation (3, 11, 29). Reduced homocysteine remethylation can result in elevated plasma homocysteine concentration, which is an independent risk factor for cardiovascular disease (19). Altered DNA metabolism is implicated in the etiology of cancers (20).

Folate serves as a metabolic carrier of one-carbon units (8). In homocysteine remethylation, folate transports methyl groups as 5-methyltetrahydrofolate (5-CH3-THF) to methionine synthase, the ubiquitous homocysteine methyltransferase that converts homocysteine to methionine in all cells (Fig. 1, reactions 6–8). Consequently, folate deficiency might alter homocysteine metabolism by limiting the availability of 5-CH3-THF for the remethylation reaction (28). The methyl groups used by methionine synthase to convert homocysteine to methionine could originate from glycine, sarcosine, dimethylglycine, histidine, formate, or the three carbon of serine (8). The latter amino acid is thought to be the primary donor of one-carbon units for homocysteine remethylation (1), and a recent human metabolic tracer study supports this hypothesis (9). Homocysteine also can be remethylated in a folate-independent manner by betaine-homocysteine methyltransferase by using betaine as a methyl donor (Fig. 1, reaction 4) (15). However, limited expression of betaine-homocysteine methyltransferase (liver and kidney only) renders most tissues dependent on methionine synthase activity for homocysteine remethylation (10, 35).

Although one-carbon metabolism is well studied in liver and kidney, relatively little is known about these pathways in mammalian intestine. Intestinal mucosa contains methionine synthase activity (21), but intestinal homocysteine remethylation capacity is restricted compared with liver and kidney due to the absence of betaine-homocysteine methyltransferase (31). This limitation renders the intestine more susceptible than liver and kidney to the homocysteine-elevating effects of folate depletion. However, the small intestine is one of only four tissues that contains both cystathionine β-synthase and γ-cystathionase, the two enzymes of the transsulfuration pathway (Fig. 1, reactions 8 and 9) (24). Transsulfuration is the only route of homocysteine catabolism, and expression of this pathway enables intestinal cells to catabolize some of the homocysteine that it produces. Whether expression of this pathway renders the intestine self-sufficient with regard to homocysteine metabolism is uncertain, because few quantitative data exist regarding the rates of one-carbon cycle reactions in this tissue.

The present investigation was designed to improve our understanding of homocysteine metabolism in intestinal cells and to assess the sensitivity of those processes to folate restriction. We hypothesized that homocysteine metabolism would consist of transsulfuration and folate-dependent remethylation and that serine would be the primary one-carbon donor for remethylation in intestinal cell cultures. Consequently, we hypothesized that homocysteine remethylation...
Homocysteine metabolism in folate-adequate and folate-deprived Caco-2 cell cultures. Homocysteine metabolism experiments were initiated by the addition of enriched medium that contained [U-13C5]methionine, [3-13C]serine, and [3H]leucine tracers at concentrations specified below to the cell cultures. The initial time course covered 24 h with time points of 0, 4, 8, and 24 h. Amino acid tracers were added to the culture medium to achieve an isotopic enrichment of ~50% for methionine, serine, leucine, and cysteine. The second time course covered 4 h with time points of 0, 0.5, 1, 2, and 4 h with amino acid tracers added to achieve an isotopic enrichment of ~20% for methionine, serine, and leucine. Cell and medium samples were taken from three culture wells at each time point.

Effects of folate restriction on homocysteine metabolism also were investigated. Cells were cultured in DMEM for days 1 and 2 post-confluence, and cultures were then divided into two groups: cells cultured in medium that contained no added folic acid (folate-restricted cultures) for days 3–7 postconfluence and cells maintained in folate-supplemented medium (control cultures) over that time course. Cells were refreshed with the appropriate medium (folate-restricted or control) 24 h before tracer experiments. Experiments were initiated by the addition of amino acid tracers ([U-13C5]methionine, [3-13C]serine, and [3H]leucine) to the medium to achieve an isotopic enrichment of ~20%. Cell and media samples were collected at 0, 0.5, 1, 2, 4, and 8 h after addition of the tracers to the media.

Collection of free intracellular amino acids. At each experimental time point, 50 μl of culture medium were collected, and cells were washed with PBS to remove remaining medium. Cells were mechanically lifted by scraping with a plastic spatula and collected in PBS (500 μl). Cell samples were acidified with 50 μl 66% (wt/vol) trichloroacetic acid and homogenized with a Polytron homogenizer. Cell homogenates were centrifuged (14,000 g for 15 min at 4°C), and the supernatants were isolated and stored at −80°C before amino acid isolation. Culture medium samples were processed and stored similarly to cell samples with the exclusion of homogenization.

To isolate free amino acids, ~500 μl of acidified supernatants were loaded onto columns packed with ~0.5 ml of AG50W-X8 cation exchange resin (Bio-Rad Laboratories) in acid form. Columns were washed with water (10 × 1 ml), followed by elution of amino acids into reaction vials by using 3 ml of 3 M NH4OH. Samples were dried in a SpeedVac (ThermoSavant, Holbrook, NY) at ambient temperature and then refrigerated overnight. Amino acids were esterified by reacting dry samples with 500 μl of a 5:1 mixture of 1-propanol/acetyl chloride and 10 μl of methanol for 1 h at 110°C. Samples were dried under N2, and the n-propyl amino acid esters were derivatized by reaction with 100 μl heptafluorobutyric anhydride and 10 μl ethanethiol for 1 h at 60°C. The reaction mixtures were dried under N2, and the resulting N-heptafluorobutyryl-n-propyl ester derivatives were solubilized in 100 μl ethyl acetate and stored at −20°C until analysis.

Isotopic enrichment was determined by gas chromatograph-mass spectrometry ( Finnigan-Thermoquest Voyager, San Jose, CA). Chromatography was performed at a helium flow rate of 1.0 ml/min using a 30-m poly(5% diphenyl/95% dimethylsiloxane)-fused silica capillary column (Equity 5; Supelco, Bellefonte, PA), using 0.3- to 2.0-μl injections and an injector temperature of 250°C. The initial oven temperature was held at 95°C for 3 min, increased to 130°C at 3.5°C/min, held at 130°C for 10 min, raised to a final temperature of 250°C at 8°C/min, and held at 250°C for 10 min. Negative chemical ionization/mass spectrometry was performed with methane as the reagent gas at a source temperature of 150°C and electron energy of 70 eV. The abundance of specific ions was determined by selected ion monitoring at the following mass/charge ratios: serine, 519–520; leucine, 349–352; methionine, 367–372; homocysteine, 549–553; and cystathionine, 678–682. Isotopic enrichments are expressed as molar ratios of labeled/nonlabeled isotopomers after correction for the natural abundance of stable isotopes (34).

Kinetic analyses. Isotopic enrichment data are plotted as the means ± SE (n = 3) at each time point. Plateau enrichments were...
determined by fitting enrichment data to single exponential curves defined by the equation $E = E_i (1 - e^{-kt})$. In this equation, $E$ is the enrichment at time $t$ (h), whereas $E_i$ and $k$ are the enrichment at infinity (i.e., plateau enrichment) and rate constant (h) from the fitted curve, respectively. Because this equation did not fit the cystathionine enrichment data of the folate restriction experiments, these data were fitted to a sigmoidal curve defined by the equation $E = E_0(1 - e^{-kt})^n$, where $c$ is an empirical constant related to the rate of change of $Y$ over time. Homocysteine remethylation and transsulfuration kinetics were calculated using data from within individual culture dishes from time points at the plateau as well as from plateau enrichments derived from curve fitting.

Behavior of the $[^{13}\text{C}]$methionine and $[^{3}\text{C}]$serine tracers in one-carbon metabolism is illustrated in Fig. 1. As the $[^{13}\text{C}]$methionine tracer is metabolized in the methylation cycle, one $^{13}\text{C}$-labeled atom is lost in the $S$-adenosylmethionine-dependent methyltransferase reactions (Fig. 1, reactions 1 and 2). Consequently, homocysteine produced from this tracer contains four $^{13}\text{C}$-labeled carbons (Fig. 1, reaction 3). Remethylation of $[^{13}\text{C}]$homocysteine with an unlabeled methyl group from 5-CH$_3$THF or betaine generates $[^{13}\text{C}]$methionine (Fig. 1, reactions 4 and 5). The fraction of the total methionine pool that had been remethylated (RM total) was calculated from enrichment data at plateau for the unmetabolized methionine pool (Ep Met $M_{\text{total}}$) and remethylated methionine (Ep Met $M_{\text{serine}}$) as

$$\text{RM total} = (\text{Ep Met } M_{\text{total}})/(\text{Ep Met } M_{\text{serine}} + \text{Ep Met } M_{\text{serine}})$$

This calculation is based on the dual tracer principle for methionine kinetics reported by Storch et al. (34).

As the $[^{3}\text{C}]$serine tracer is metabolized in the folate cycle, the $^{13}\text{C}$-labeled three carbon is transferred to tetrahydrofolate (Fig. 1, reaction 6), reduced by MTHFR (Fig. 1, reaction 7), and subsequently transferred to homocysteine via methionine synthase to generate $[^{13}\text{C}]$cystathionine (Fig. 1, reaction 8). Cleavage of $[^{13}\text{C}]$cystathionine yields $[^{13}\text{C}]$cysteine and $\alpha$-ketobutyrate (Fig. 1, reaction 9). The percentage of the cysteine pool produced by transsulfuration ($\%\text{Cys from TS}$) and the percentage of the cysteine pool produced by the folate enzymes are calculated by using the plateau enrichments of $[^{13}\text{C}]$cystathionine, $[^{13}\text{C}]$cysteine (Ep Cys $M_{\text{serine}}$), and Ep Ser $M_{\text{serine}}$ as

$$\%\text{Cys from TS} = (\text{Ep Cys } M_{\text{serine}}/\text{Ep Cys } M_{\text{serine}}) \times 100$$

and

$$\%\text{Cys} = (\text{Ep Cys } M_{\text{serine}}/\text{Ep Cys } M_{\text{serine}}) \times 100$$

Data were analyzed by $t$-test or two-way ANOVA (17) with folate concentration and incubation time as the main factors. Multiple comparisons were conducted using the Student-Newman-Keuls method. All data are presented as means $\pm$ SE.

RESULTS

Homocysteine metabolism in folate-adequate Caco-2 cell cultures. Plots of isotopic enrichment vs. time for intracellular amino acids in 24- and 4-h time courses indicated that labeled intracellular precursor pools reached plateau enrichment at $\sim 1$ h posttreatment (Figs. 2 and 3). Plateau enrichments derived by fitting data to a single exponential curve are presented in Table 1. The fraction of the intracellular free methionine pool formed by remethylation of homocysteine was $18 \pm 0.12$ and $15 \pm 0.13\%$ in the 4 h (1–4 h data combined; $n = 9$) and 24 h (4–24 h data combined; $n = 9$) time courses, respectively. The fraction of the intracellular free methionine pool that was formed through folate-dependent remethylation using the three carbon of serine was $19 \pm 0.54$ and $12 \pm 0.26\%$ in the 4 h (2- and 4-h data combined; $n = 6$) and the 24 h (4- 24-h data combined; $n = 9$) time courses, respectively. Consequently, serine provided 108 and 82% of the one-carbon units used for remethylation in the 4- and 24-h cell culture experiments, respectively.
Table 1. Plateau enrichments of stable isotope-labeled amino acids in Caco-2 cell cultures after being cultured in medium containing \([^{13}C_5]\)methionine and \([^{3,13}C]\)serine

<table>
<thead>
<tr>
<th>Labeled Amino Acid</th>
<th>Plateau Enrichment, mol %excess</th>
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<tbody>
<tr>
<td></td>
<td>Trial A</td>
</tr>
<tr>
<td>([^{3}H])leucine</td>
<td>16.6 ± 0.846</td>
</tr>
<tr>
<td>([^{1}C])serine</td>
<td>10.5 ± 0.542</td>
</tr>
<tr>
<td>([^{1}C])methionine</td>
<td>2.38 ± 0.278</td>
</tr>
<tr>
<td>([^{1}C])cystathionine</td>
<td>9.99 ± 1.30</td>
</tr>
<tr>
<td>([^{1}C])cysteine</td>
<td>1.51 ± 0.121</td>
</tr>
<tr>
<td>([^{3}H])cysteine</td>
<td>12.8 ± 1.86</td>
</tr>
<tr>
<td>([^{1}C])methionine</td>
<td>15.7 ± 0.108</td>
</tr>
<tr>
<td>([^{1}C])cystathionine</td>
<td>3.40 ± 0.0229</td>
</tr>
<tr>
<td>([^{1}C])homocysteine</td>
<td>11.0 ± 0.904</td>
</tr>
<tr>
<td>([^{1}C])cystathionine</td>
<td>10.9 ± 1.55</td>
</tr>
</tbody>
</table>

Data (means ± SE) are derived by fitting time course data to a single exponential curve. See MATERIALS AND METHODS for details. *Trial A*, 20% initial enrichment, 4-h time course; *Trial B*, 50% initial enrichment, 24-h time course.

Effects of folate deprivation on homocysteine metabolism in Caco-2 cell cultures. Folate restriction did not affect the enrichment patterns of \([^{3,13}C]\)serine or \([^{13}C_5]\)methionine tracers (Fig. 4). However, significantly reduced enrichments of \([^{13}C_1]\)methionine \((P < 0.001\) for individual 1- to 4-h time points, \(P < 0.005\) for plateau enrichments derived from curve-fitting), \([^{13}C_4]\)methionine \((P < 0.01\) for individual 1- to 4-h time points, \(P = 0.02\) for plateau enrichments derived from curve-fitting), and \([^{13}C_4]\)cystathionine \((P < 0.001\) for individual 1- to 4-h time points, nonsignificant \((P = 0.08\) for plateau enrichments derived from curve-fitting) were measured in folate-restricted cultures (Table 2).

(Discussion)

Previous studies demonstrated that the activities of enzymes of the methionine cycle (21, 24) and the transsulfuration pathway (7, 24) are present in isolated rat enterocytes and rat intestinal mucosa. To the best of our knowledge, this is the first investigation of the kinetics of the homocysteine remethylation and transsulfuration pathways in an intestinal cell model of any species. This report also contains the rst direct evidence that folate deprivation alters homocysteine metabolism in these cells. Because the Caco-2 cell model displays many characteristics of absorptive intestinal cells, similar results are expected in mammalian enterocytes and intestinal mucosa.

An assumption of this tracer model is that all of the \([^{13}C_1]\)methionine measured is produced from remethylation of homocysteine with one-carbon units derived from...
[3-13C]serine. This may be overestimation, because the metabolism of the [U-13C5]methionine tracer via the choline synthesis/degradation pathways and polyamine synthesis pathway followed by recycling of a labeled methyl group and its subsequent use for remethylation can also yield [13C1]methionine. These pathways are not believed to be quantitatively significant in overall methionine metabolism, however, and are ignored in this tracer model. The importance of these alternative pathways of [13C1]methionine metabolism is the subject of a current investigation in our laboratory.

Remethylated homocysteine comprised a significant fraction (18–19%) of the free methionine pool in the Caco-2 cell cultures, although exogenous methionine was accessible in the culture medium. These results indicate the importance of the methylation cycle to methionine metabolism in Caco-2 cell cultures. Significant remethylation activity is understandable in this cell type as a consequence of the sizable methionine and cysteine requirements of the intestine for protein synthesis, as well as for maintenance of methylation reactions in tissues with a large cell turnover rate.

Reaction pathways that generate one-carbon units from betaine, dimethylglycine, sarcosine, glycine, and histidine are present in the liver and kidney (8). Although these compounds have been implicated as significant contributors of one-carbon groups for remethylation (26, 27, 37, 39), the three carbon of serine provided nearly all of the one-carbon units for homocysteine remethylation in folate-adequate Caco-2 cells (Figs. 2 and 5C). This result was predictable, considering that the intestine does not express betaine-homocysteine methyltransferase, has little expression of glycine N-methyltransferase or the glycine cleavage system, and is not known to express the enzymes of histidine catabolism (31, 38, 39). A similar dependence of one-carbon metabolism on serine might exist in most tissues other than the liver and kidney. For example, the three carbon of serine accounted for 95% of one-carbon groups in the human MCF-7 breast cancer cell line, whereas one-carbon group production from glycine cleavage was undetectable (14). Furthermore, >80% of one-carbon units used for total body remethylation were estimated to be derived from the three carbon of serine in our recent studies (9) with healthy humans, which suggest that tissues other than the liver and kidney

![Fig. 4. Enrichment curves of labeled amino acids from control of folate-restricted Caco-2 cell cultures. A: [3-13C]serine tracer-derived enrichment curves from control cultures. B: [U-13C5]methionine tracer-derived enrichment curves from control cultures. C: [3-13C]serine tracer-derived enrichment curves from folate-restricted cultures. D: [U-13C5]methionine tracer-derived enrichment curves from folate-restricted cultures. Data are presented as means ± SE (n = 3).](http://ajpgi.physiology.org/)

Table 2. Plateau enrichments of stable isotope-labeled amino acids in Caco-2 cells cultured in medium with or without supplemental folate (control and folate-restricted, respectively) for 4 days followed by incubation in culture medium containing [U-13C5]methionine and [3-13C]serine

<table>
<thead>
<tr>
<th>Labeled Amino Acid</th>
<th>Plateau Enrichment, mol % excess</th>
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<tbody>
<tr>
<td></td>
<td>Supplemented</td>
</tr>
<tr>
<td>[2H3]leucine*</td>
<td>11.6 ± 0.503</td>
</tr>
<tr>
<td>[13C5]serine*</td>
<td>8.22 ± 0.841</td>
</tr>
<tr>
<td>[13C5]methionine*</td>
<td>2.04 ± 0.252</td>
</tr>
<tr>
<td>[13C4]cystathionine†</td>
<td>9.21 ± 1.16</td>
</tr>
<tr>
<td>[13C5]methionine*</td>
<td>10.8 ± 0.708</td>
</tr>
<tr>
<td>[13C4]homocysteine*</td>
<td>11.4 ± 1.72</td>
</tr>
<tr>
<td>[13C5]methionine*</td>
<td>2.62 ± 0.194</td>
</tr>
<tr>
<td>[13C4]cystathionine†</td>
<td>11.6 ± 0.810</td>
</tr>
</tbody>
</table>

*Data (means ± SE) are derived by fitting time course data from Fig. 4 to a single exponential curve. See MATERIALS AND METHODS for details. †Data (means ± SE) are derived by fitting time course data from Fig. 4 to a sigmoidal curve. See MATERIALS AND METHODS for details. ‡Significantly different from folate-adequate cultures; P < 0.05.
primarily depend on serine to maintain remethylation. It seems plausible that the 10–20% of the one-carbon units used for total body remethylation that were not derived from the three carbon of serine were produced in the liver and kidney from the breakdown of choline, histidine, and glycine.

It has been known for decades that folate restriction alters one-carbon metabolism and specifically the use of the three carbon of serine in one-carbon reactions (2). However, little is published on the effects of folate restriction on one-carbon metabolism in the intestine. Folate deficiency can reduce the colonic S-adenosylmethionine-to-S-adenosylhomocysteine ratio (30), and folate depletion alters certain aspects of DNA metabolism related to the one-carbon cycle (6, 11). This is the first report of reduced total remethylation (~33%) and reduced folate-dependent remethylation from serine (~60%) caused by folate restriction (Fig. 5, A and B).

The ~50% of unaccountable remethylation in folate-restricted cultures (i.e., not derived from the three carbon of serine) argues that one or more of the normally minor one-carbon donor pathways was more active under conditions of folate inadequacy than folate adequacy. Under folate-deficient conditions, glycine, histidine, betaine, dimethylglycine, and sarcosine may become important in intestinal one-carbon metabolism. This seems counterintuitive, because betaine-homocysteine methyltransferase is not expressed in intestine, and all other donors also require folate for their use in one-carbon metabolism. It is possible, however, that one or more of these pathways is more resistant to folate deficiency than the serine-dependent pathway. For example, histidine was estimated to provide ~20% of the one-carbon units for total body remethylation in rats, and its use was not hindered in folate deficiency (27). An alternative explanation for the unaccountable one-carbon donation to remethylation is that this cell line might express the enzymes of one-carbon metabolism in a pattern different from intact intestine or intestinal mucosa.

Other than a report that the transsulfuration pathway has a role in cysteine metabolism of isolated rat enterocytes (7), the significance of the transsulfuration pathway’s presence in the intestine is not well defined. The transsulfuration flux observed in Caco-2 cells was greater than anticipated based on in vitro
mucosal cystathionine β-synthase and γ-cystathionase enzyme activities relative to those observed in kidney and liver (24). We found transsulfuration to be quantitatively significant in homocysteine catabolism and cysteine production (13% of cysteine flux) in Caco-2 cells. Finkelstein (12) suggested that tissues containing the transsulfuration pathway enzymes utilize this pathway for cysteine production in support of their large demands for glutathione. In support of this hypothesis, transsulfuration produced nearly half of the cysteine and was used for a significant fraction of glutathione production in the HepG2 human hepatoma cell line, and transsulfuration flux was induced under oxidative conditions that consume glutathione (23). Increased transsulfuration fluxes also could be a function of the high methionine content of the medium, because subsequent overproduction of S-adenosylmethionine might activate the transsulfuration pathway. However, there is little evidence that upregulation of transsulfuration flux by methionine occurs in extrahepatic tissues (12).

A functional transsulfuration system could empower the intestine to assist the liver in the catabolism of homocysteine, similar to the role of the kidney (4). Homocysteine removal capacity of the intestine might be less significant than that of the kidneys, however, because the activities of the transsulfuration pathway enzymes of rat mucosa in vitro were ~20–40% of that found in rat kidney (24). As yet, there is no evidence that the intestine imports homocysteine from the plasma, but the high transsulfuration activity in this organ suggests that it could assist in homocysteine catabolism. In contrast, the discovery that media recovered from Caco-2 cell cultures contained [13C]homocysteine and [13C]cystathionine, which are produced intracellularly from the methionine and serine tracers, suggests that the intestine might contribute to the plasma homocysteine load. It also suggests that the transsulfuration pathway may not have the capacity to handle excess homocysteine, which it might produce. More research is needed to determine the role of the intestine in regulating the homocysteine concentration of the plasma.

Reduced labeling of cystathionine in Caco-2 cells cultured in low-folate medium could be interpreted in two ways. If the cystathionine pool size of the folate-restricted cell cultures was similar to or smaller than that found in control cultures, then these data suggest that transsulfuration flux was reduced in folate restriction. This could be due to an overall reduction of methylation cycle flux in these cells, but the similar rates of homocysteine labeling between folate-restricted and control cells argue against this possibility. An alternative interpretation is that the labeled cystathionine produced under these conditions is diluted in a greater overall cystathionine pool size in folate-restricted cultures. An argument for this possibility is that folate restriction is associated with elevated plasma cystathionine concentrations in humans and rats (25, 32, 33) and might indicate that transsulfuration pathway activity is elevated in response to reduced remethylation capacity. Without knowledge of the intracellular cystathionine pool size, however, we cannot ascertain the mechanism behind the slower labeling of the cystathionine pool with folate restriction at this time.

In conclusion, with the combined [3,13C]serine and [U-13C]methionine tracer model we found significant activity of the remethylation and transsulfuration pathways in the Caco-2 model of absorptive intestinal cells. The three carbon of serine provides nearly all of the one-carbon units used for remethylation in folate-supplemented cultures. Remethylation was reduced with folate restriction, and under these conditions the three carbon of serine provided only ~50% of the one-carbon units used for remethylation. Transsulfuration flux was significant and provided 13% of cellular cysteine in folate-supplemented Caco-2 cell cultures. Export of homocysteine and cystathionine from these cells might indicate that the intestine contributes to the plasma load of these metabolites, although these cells can catabolize them via transsulfuration. Further studies are needed to determine the importance and quantitative significance of the donors of one-carbon groups for remethylation during folate restriction as well as to determine whether intestinal cells have an impact on the plasma homocysteine load.

GRANTS
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